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VOL. 7 æ 1955 æ NO. 1

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The Scandinavian Journal of Clinical and Laboratory Investigation is edited for the Scan navian Society for Clinical Chemistry and Clinical Physiology, and will ap quarterly, 4 issues forming a volume. Subscription price N. Kr. 35.00 per volume in the Scandina countries. Other countries Kr. 50.00.

The Scandinavian Journal of Clinical and Laboratory Investigation publishes original arti closely associated with clinical research and based on laboratory investigations. Pure methodolo work of importance for clinical research will also be accepted for publication. Authors in the Sca navian countries should send their manuscripts to one of the editors in their respective countries. T addresses are printed above.

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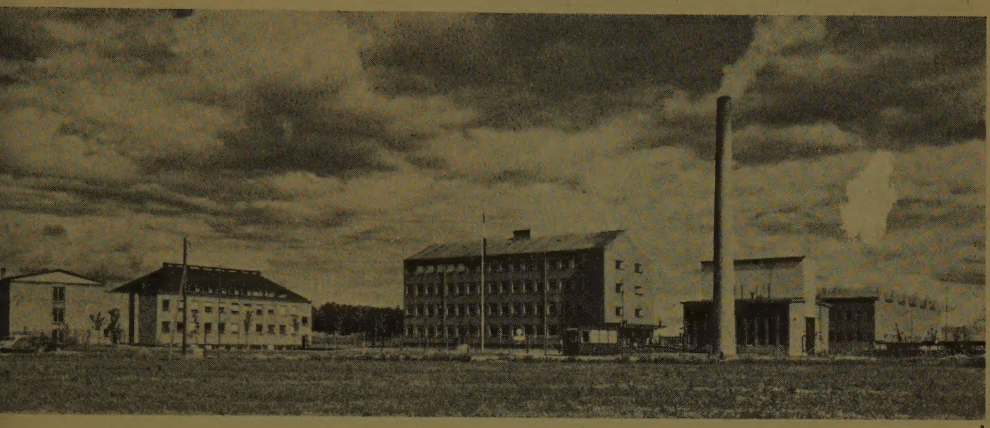

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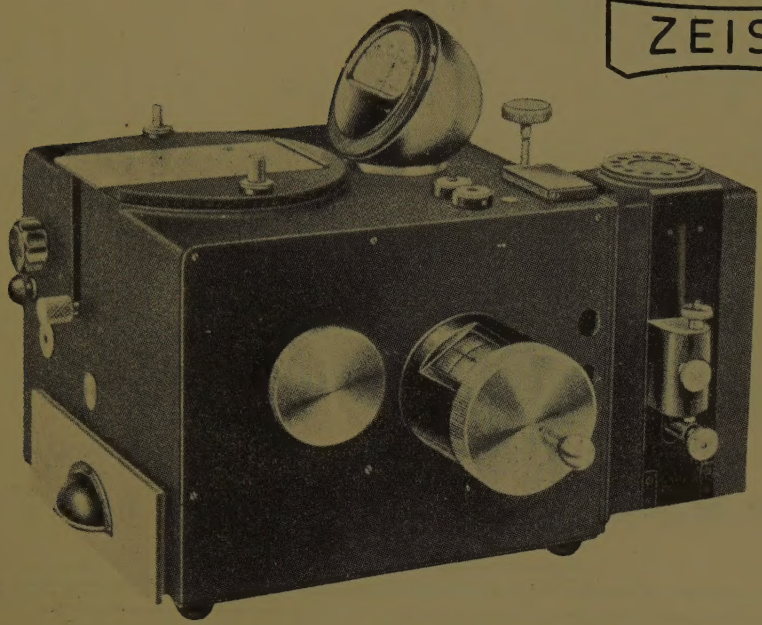

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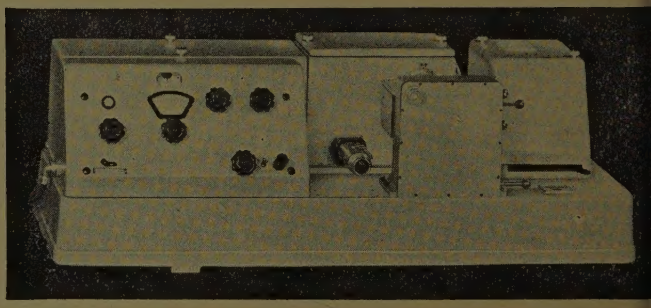

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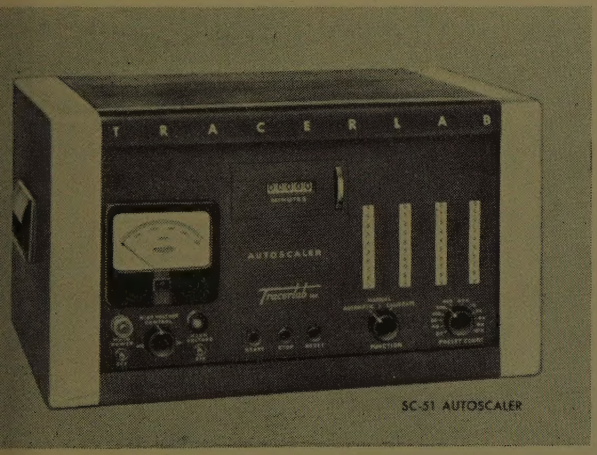

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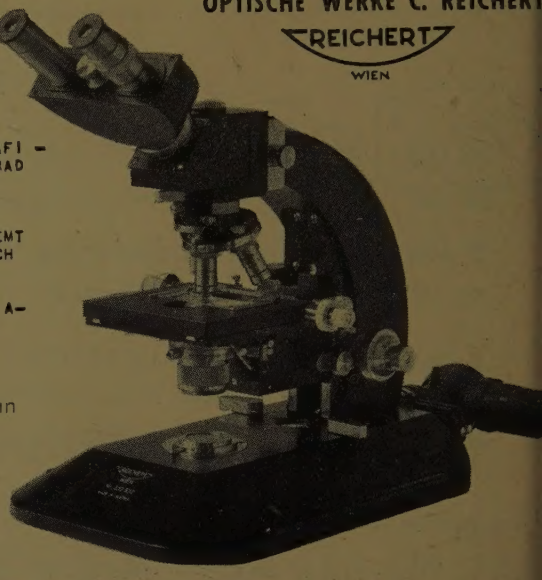

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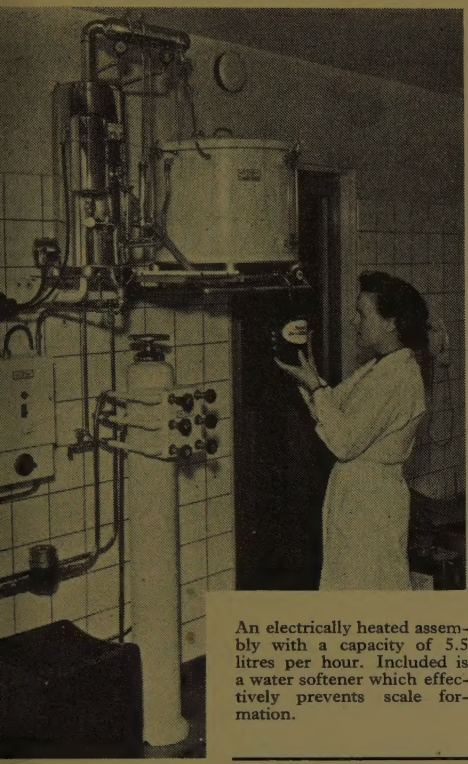

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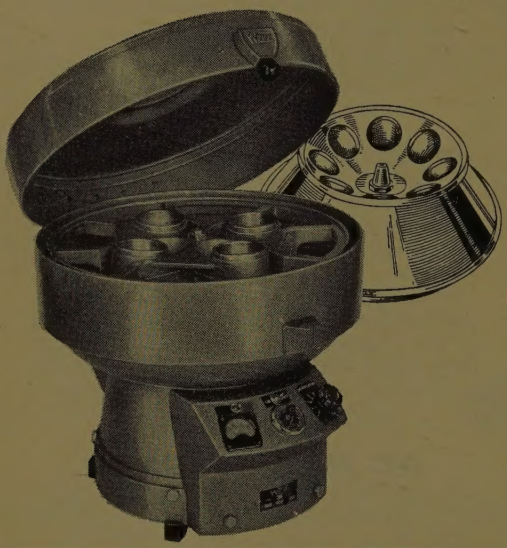

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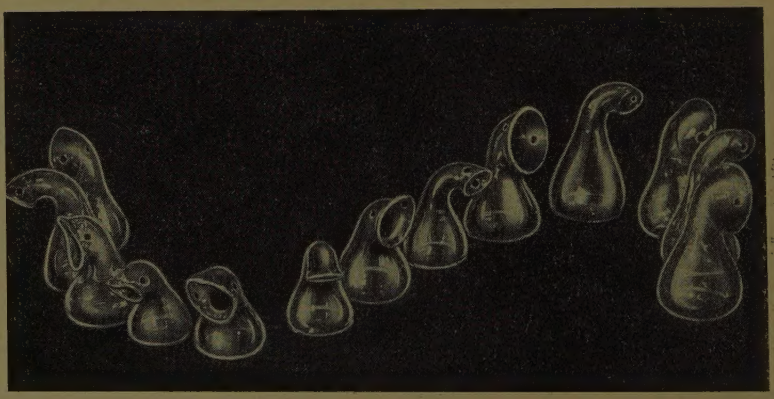

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NORADRENALINE AND ADRENALINE IN THE URINE
PART I. THEIR CHEMICAL DETERMINATION

By A. PEKKARINEN and M-E. PITKÄNEN

From the Department of Medical Chemistry, University of Helsinki, Finland

(Received for publication Jan. 21, 1954)

several decades attempts have been to determine the adrenaline content blood as an estimation of sympathetic us system activity in the organism. uman peripheral blood the normal nt of adrenaline and noradrenaline, ver, is very small (Pekkarinen, Lund, 1951, Weil-Malherbe, 1953). e case of tumor containing adrenaline noradrenaline, in phaeochromocytoma aranganglioma, small concentrations been demonstrated in the peripheral during a hypertensive phase, from 9.8 μg per cent, consisting nearly all oradrenaline (Lund & Møller, 1951, 1952). Adrenaline and noradrenaline fective in extremely small doses, which ore can hardly be demonstrated in the ation, and can produce pronounced ological changes in the organism (Pek- en & Hortling, 1951, Helve & Pekka- 1952, Pekkarinen & Aro, 1952). after intravenous infusion of very large doses they disappear rapidly from the circulation (Pekkarinen, 1948, Lund, 1951).

Several hormones, such as certain steroids, or their metabolites, can be more easily detected in the urine than in blood. The same is true for the sympathetic hormones, adrenaline and noradrenaline. Adrenaline, given in large oral doses or as an infusion, appears in the urine partly in combination with sulphuric acid, glucuronic acid or with phenols. After hydrolysis it causes positive chemical or biological tests (Falta & Ivcovic, 1909, Weinstein & Manning, 1937, Richter, 1940, Richter & MacIntosh, 1941, Beyer & Shapiro, 1945, Bacq, Lecomte & Fischer, 1949, Clark, Akawie, Pogrund & Geissman, 1950). However, the chemical tests, earlier used, are not sensitive enough for the detection of the small amounts of adrenaline and noradrenaline normally excreted in the urine.

In recent years we have seen an advance in the ability to diagnose adrenal medullary tumours, due principally to biological

methods developed for the determination of adrenaline and noradrenaline in the urine (Engel & v. Euler, 1950, v. Euler & Hellner, 1951, 1952, Goldenberg & Rapport, 1951, Carl, Hildebrandt & Marquardt, 1951, Holtz, Kroneberg & Schümann, 1950) as well as pharmacological tests such as the histamine (Roth & Kvale, 1945), dibenamine (Spear & Griswold, 1948, Cahill & Monteith, 1951, Bierman & Partridge, 1951), benzodioxane (Goldenberg, Harrison & Brown, 1947, Goldenberg, Aranow, Smith & Faber, 1950, Calkins, Dana & Howard, 1951, Faber, 1951) and regitine tests (Emlet, Grimson, Bell & Orgain, 1951, Grifford, Roth & Kvale, 1952). Many cases of phaeochromocytoma have been intensively studied by pharmacological tests (Smithwick, Greer, Robertson & Wilkin, 1950, Vries, Rachmilewitz & Schumert, 1949, Gulbertson, Greer, Halperin, Litter, Burnett & Smithwick, 1949, Allen, Cannon, Upson, Huizenga, Bastron & Waugh, 1951, Riishede, 1951). Biological and pharmacological tests as aids in diagnosis have greatly enhanced accuracy in detecting their presence.

Few investigations of adrenaline and noradrenaline in the urine by chemical methods have been made until the present, and these on subjects who have received relatively excessive amounts of adrenaline orally, or in experimental animals receiving infusions. It is therefore desirable to develop a chemical method for the determination of small amounts of these substances in the urine.

OWN INVESTIGATIONS

The general procedure involved has been published as a preliminary report in a previous paper (Pekkarinen & Pitkänen, 1951). In the development of this me it was realized early that there were se facts to be overcome in the urine: 1) interferring fluorescing substances; 2) ous inhibiting and reducing substa 3) undesirable precipitation of various stances such as calcium and phosph during the fluorescence reaction after a tion of alkali; 4) differentiation of fluorescence reactions due to adrenaline noradrenaline.

During concentration in *vacuo* of solut obtained from urine as used earlier in logical methods, reducing compounds as ascorbic acid were also concentrated often completely inhibited the fluores reaction of adrenaline. Again, simultan concentration of other fluorescing substa interfered with the specificity of the reac

In the chromatographic adsorption an aluminum oxide column (Lund, 1 there was difficulty of maintaining no flow through columns in the presen precipitated phosphates at pH 8.5. — Mi of aluminum oxide and urine sample blendor at pH 8.5, followed by centrifug and several washings, was found to be factory for the adsorption of adrenaline noradrenaline and for the eliminatio interferring precipitates fluorescing reducing substances.

METHOD

1) A 200 ml or an aliquot of the 24 hour spe of urine, which has been collected in acid so (PH 4), is brought to pH 8.5 with 1 N s hydroxide.

2) Precipitated phosphates are centrifuge filtered off.

3) Adrenaline and noradrenaline are ads by addition of 8 g of aluminum oxide, designe

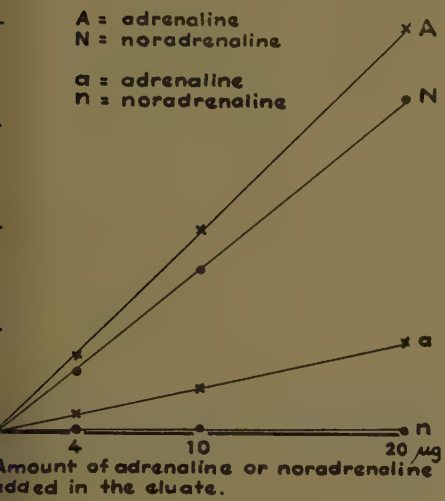

Fig. 1.

tographic analysis (a ratio of aluminum solution volume of 1 :25), followed by tes mixing with blendor and centrifugation. The supernatant fluid is decanted and the um oxide precipitate is washed 3—5 times 00 ml portions of distilled water by centri- n.

Adrenaline and noradrenaline are eluted twice ml of 1 N oxalic acid (1/10 of urine volume) ntrifuged. Oxalic acid precipitates also the salts which after standing some hours are ged off.

The pH of the eluate is increased to 6.3 with dium hydroxide and stabilized by 2 N sodium buffer, and the solution brought to a con- volume. The eluate has been normally 10 times with distilled water before the on, because an inhibition of the fluorescence has been encountered in the oxalic acid without dilution. To three aliquots are added ively, distilled water, adrenaline standard radrenaline standard.

Adrenaline and noradrenaline are oxidised eno- and noradrenochrome by mixing 10 ms of manganese dioxide to each 6 ml of these three samples for 30 seconds. lutions are then centrifuged and carefully filtered to remove all particles of manganese dioxide.

8) In 2 ml of these three filtrates the fluorescence reaction is developed in alkaline solution by the addition of 0.4 ml of 5 N sodium hydroxide containing 200 mg per cent of freshly prepared ascorbic acid and determined in Farrand photoelectric fluorometer (Corner Filter no. s. 5860 and 3389). To three 2 ml aliquots serving as blank respectively the same amount of alkali is added without ascorbic acid. —

9) For the differentiation of noradrenaline from adrenaline in the eluate, the fluorescence reaction is simultaneously performed without manganese dioxide oxidation by direct addition of alkali without ascorbic acid, as follows: to 2 ml of each of the remaining unoxidised aliquots of eluate and standards are added 0.4 ml of 5 N sodium hydroxide. The fluorescence reaction of noradrenaline develops later and is much less intense than the adrenaline fluorescence reaction, which appears first. The slowly developing noradrenaline fluorescence at the time of maximal adrenaline fluorescence is very weak, being only 1 to 2 per cent of the amount observed after oxidation, and 25 per cent of the amount observed after adrenaline fluorescence. (Fig. 1.)

10) Recovery of adrenaline and noradrenaline added to the sample has been 75—80 per cent. (Fig. 2.)

11) For the determination of conjugated adrenaline and noradrenaline, simultaneous estimation of these substances has been made after hydrolysis of urine samples by boiling for 30 minutes at pH 1.5.

The results observed in excretion of adrenaline and noradrenaline in certain normal and pathological conditions are published in the next paper (Pekkarinen & Pitkänen, 1954)).

DISCUSSION

Collection of the 24 hour urine specimen should be done at pH 4 to inhibit the oxidation of adrenaline and noradrenaline. Extra amounts of acid should be avoided since the large concentrations of salts so forced is likely to inhibit the effective adsorption of

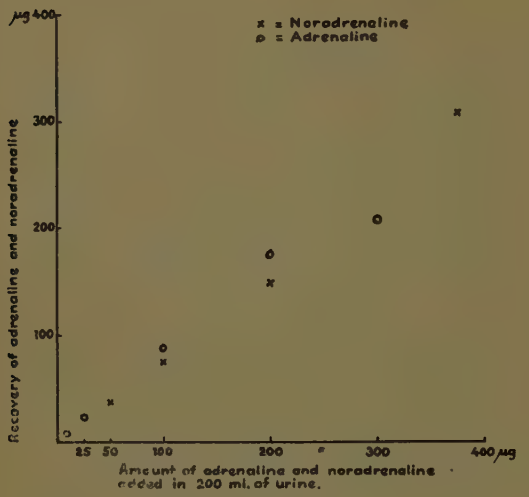

Fig. 2.

adrenaline and noradrenaline onto aluminum oxide at pH 8.5. Furthermore, if the pH of the urine sample is not reduced from neutral or alkaline reaction to 4, a fraction of adrenaline and noradrenaline will be oxidised during the collection. In phaeochromocytoma with paroxysmal hypertension, the collection of urine should start immediately after an attack and continue for 24 hours thereafter. In cases of continuous elevation of blood pressure without attacks, the time of collection is of less importance.

The urine contains large quantities of soluble salts such as phosphates and calcium, which may interfere as precipitates in the estimation of the fluorescence reaction at an alkaline pH. Before the adsorption of adrenaline and noradrenaline onto aluminum oxide, phosphates, which precipitate at pH 8.5, are washed well or centrifuged off. Calcium salts are removed as oxalates after the elution with oxalic acid.

The adsorption at pH 8.5, used earlier adrenaline and noradrenaline (Pekkari 1948, Lund, 1951, Pekkarinen & Pitkä 1951), has been found to be satisfactory in urine. The best types of aluminum o for quantitative adsorption are those desi for chromatographic analysis. We have relatively large amounts of aluminum o in the urine sample (a ratio 1 : 25), bec the adsorption of adrenaline and noradr line is then complete. To inhibit the o tion of small concentrations, especiall adrenaline, but also of noradrenalin pH 8.5 during adsorption and washi delay in the processing of samples is avoided. It is possible that at higher in biological methods, adrenaline may some biological activity, though this i true for noradrenaline, which is more s toward alkaline oxidation. However, is no evidence of destruction of adren in the fluorescence reaction as used probably due to the short time of exp of these substances to a pH of 8.5. PH earlier in biological determinations, in opinion, in urine might be too low for mum adsorption.

Washing with large amounts of dis water is necessary for the eliminatio other fluorescing and inhibiting substa which otherwise may disturb the flu cence reaction of adrenaline and nor naline. Washing does not elute adren and noradrenaline at pH 8.5. Also, if n sary, it is possible to use a buffer of 0. sodium acetate-sodiumhydroxide, pH It is necessary to decant the washings fully as the part of the precipitate is fine.

o elutions with 1 N oxalic acid has
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ry of adrenaline and noradrenaline
aluminum oxide. It also precipitates
n salts and does not dissolve aluminum
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the determination of adrenaline and
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manganese dioxide treatment is used
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onditions under which adrenaline and
renaline in the eluate yield a fluores-
reaction after manganese dioxide
tion and addition of alkali with ascorbic
must be carefully maintained. The pH
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checked. An extra amount of manga-
lioxide should be avoided, and the man-
e dioxide particles after oxidation care-
centrifuged and filtered. Excess
nts of manganese dioxide can cause
her oxidation of adreno- and noradre-
ome and, consequently, result in
ase in the fluorescence reaction.

e eluate must not contain ascorbic acid
to oxidation with manganese dioxide,
ascorbic acid in the concentration of 10
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nces, adreno- and noradrenolutine. On
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abilization of the fluorescence reaction
aline solution after manganese dioxide
tion. When, as a specificity test, alkali
led without ascorbic acid in the blank,
the adreno- and noradrenochrome are rapidly oxidised to melanin pigments, and the fluorescence reaction then disappears rapidly and only the fluorescence due to the blank remains, which is normally small.

To differentiate between adrenaline and noradrenaline in the eluate we have used the simultaneous determination of the fluorescence reaction of adrenaline and noradrenaline after addition of alkali directly, instead of manganese dioxide oxidation at pH 3. When the standard fluorescence reactions of the urine eluate are known with and without manganese dioxide oxidation, the amounts of adrenaline and noradrenaline present in the sample can be determined. However, in normal urine eluate, the fluorescence reaction without manganese dioxide oxidation is very small, when the relative amount of noradrenaline as compared to adrenaline is large. Therefore the differentiation of the amount of adrenaline and noradrenaline in the eluate is easier in the presence of increased urinary excretion of noradrenaline and adrenaline as in cases of pheochromocytoma.

SUMMARY

A method has been described for the determination of adrenaline and noradrenaline in urine based on adsorption at pH 8.5, by mixing with aluminum oxide and centrifugation, elution by oxalic acid and on the fluorescence reaction after manganese dioxide oxidation. Other fluorescing and inhibiting substances are eliminated by washing the aluminum oxide several times with distilled water; phosphates are eliminated at pH 8.5, and calcium salts during the oxalic acid

eluation. The method for the differentiation of adrenaline and noradrenaline in the urine eluate has been described. 75 to 80 per cent of adrenaline and noradrenaline can be recovered.

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NORADRENALINE AND ADRENALINE IN THE URINE. PART II. THEIR EXCRETION IN CERTAIN NORMAL AND PATHOLOGICAL CONDITIONS

By A. PEKKARINEN and M-E. PITKÄNEN

From the Department of Medical Chemistry, University of Helsinki, Helsinki, Finland

(Received for publication Jan. 21, 1954)

By means of the fluorescence reaction (Pekkarinen & Pitkänen, 1954) we have studied the daily urinary excretion of adrenaline and noradrenaline in normal and certain pathological conditions, and in surgical patients after intravenous infusion of noradrenaline.

Excretion in normal individuals (Table I)

The mean of the daily excretion of noradrenaline and adrenaline (as noradrenaline equivalent) has been 81 μg in 17 medical students maintaining normal daily activity. Excretion varied between 31 and 185 μg per 24 hours. The concentration ranges in the urine have been between 3 and 17 μg per cent.

Excretion in certain pathological conditions (Table II)

In 26 patients, mainly cases of hypertension, and a few patients with neurosis clinically suspected of having phaeochromocytoma, clearly elevated excretion in the urine has been found in 3 cases. One of them had a continually increased excretion of noradrenaline and one showed marked elevation only during one day.

In the first patient we determined the daily excretion during 7 days in a period of two weeks. During this time the excretion was 1354 μg per day (r from 430 to 2400 μg). The concentr of noradrenaline in the urine was markedly increased, the average being per cent. The ranges of the concentra were from 29 to 150 μg per cent. The e tion was totally noradrenaline; no adre excretion was observed.

Table I. *Excretion of noradrenaline the urine in normal individuals duri adrenaline (as noradrenaline equivalen daily activity.*

Urine volume per 24 hrs. ml	Amount of noradrenaline μg 24 hrs	Concentrat noradrenal the urine cent
650	38	5.8
700	52	7.4
950	162	17.0
750	46	6.0
850	50	6.0
570	59	10.0
1000	130	13.0
650	80	12.0
850	37	4.0
1600	73	6.0
400	31	8.0
800	95	12.0
2300	79	3.0
750	55	7.0
1400	185	13.0
1400	130	9.3
800	78	10.0
Average 954	81	8.8

le II. *Excretion of noradrenaline and adrenaline (as noradrenaline equivalent) urine in patients with a noradrenaline-secreting tumor, with hypertension, neuroses, and other conditions.*

Patient No.	Date	Urine volume per 24 hrs. ml	Excretion of noradrenaline per 24 hrs.		Concentration of noradrenaline in the urine µg per cent	Clinical Diagnosis	Blood pressure mm Hg
			Free µg	Conjugated µg			
1	30.5.52	1450	430	520	30	Paraganglioma of the abdominal aorta	310/140
	5.6.52	1900	650	510	34		
	6.6.52	1750	1040	470	108		
	7.6.52	1300	1400	1720	108		
	11.6.52	2100	1450		69		
	12.6.52	1950	2110		108		
	13.6.52	1600	2400		150		
	average	**1720**	**1354**		**80**		
2	30.5.52	1075	244		23	Status post nephritidem	240/150
	31.5.52	1250	590		47		
	8.6.52	1350	480		35		
	9.6.52	750	550		70		
	10.6.52	1000	150		15		
	11.6.52	1550	110		7		
	20.7.52	1200	54		4.5		
	21.7.52	1050	47		4.5		
	average	**1160**	**280**		**26**		
3	21.10.51	2500	180		7	Phaeochromocytoma?	140/100
	22.10.51	2400	1390		58		
	23.10.51	2000	190		10		
	5.11.51	2020	150		7		
	11.2.52	3100	230		8		
	21.3.52	2650	80		3		
	average	**2445**	**375**		**15.5**		
4	2.10.51	800	96		12	Ureter et pelvis renis duplex lat. sin. et hypertensio arterialis nephrogenea. Resectio renis sin.	220/110
	3.10.51	400	19		5		
	4.10.51	600	85		14		
	average	**600**	**66**		**10**		
5	21.9.51	800	31		4	Arterial Hypertension. Status post sympathectomiam lumb. et thor.lat.-dx. Ren.duplex dx.	210/140
	22.9.51	450	28		6		
	average	**625**	**30**		**5**		
6	8.4.52	800	124	8	12	Aneurysm of the abdominal aorta. Hypertension.	235/130
	16.4.52	1400	110		8		
	19.4.52	1200	36	4	3		
	20.4.52	1100	145	35	13		
	26.4.52	450	61	45	14		
	average	**990**	**95**		**10**		

Age	Sex.	Patient No.	Date	Urine volume per 24 hrs. ml	Excretion of noradrenaline per 24 hrs.		Concentration of noradrenaline in the urine µg per cent	Clinical Diagnosis	Bl pres mm
					Free µg	Con-ju-gated µg			
26	♀	7	26.3.52	900	173	87	19	Malignant hypertension.	200
			20.5.52	1900	100	35	5		
			average	**1400**	**137**		**12**		
49	♂	8	20.5.52	700	158	0	23	Malignant hypertension.	205
			29.5.52	800	184		24		
			30.5.52	1000	115		12		
			average	**833**	**152**		**20**		
42	♀	9	31.3.52	1040	140		13	Malignant hypertension.	290
22		10	21.3.52	800	130		16	Hypertension.	230
			22.3.52	500	90		18		
50	♂	11	25.5.52	700	99	17	14	Hypertension. Carcinoma of the stomach with liver metastases.	210
25	♀	12	3.6.52	750	73		10	Hypertension.	260
32	♂	13	24.9.51	800	72		9	Malignant hypertension.	225
56	♀	14	18.2.52	600	30		5	Essential hypertension.	220
			19.2.52	750	41		6		
21	♂	15	20.4.52	400	27		7	Essential hypertension.	200
			21.4.52	700	25		4		
			22.4.52	400	33		5		
43	♂	16	3.6.52	800	200		25	Neurosis.	135
33	♂	17	11.1.52	1200	135		11	Pheochromocytoma ?	165
			11.3.52	900		76	8		
40	♀	18	27.10.51	1425	77		5.4	Hyperinsulinismus.	130
28	♂	19	11.2.52	2100	62		3	Neurocirculatory dystonia.	170
			12.2.52	1850	68		3.1		
18	♀	20	14.2.52	1500	66	66	4.4	Psychoneurosis.	165
		21	7.2.52	1500	59		3.9	Tuberculosis pulmonum.	
23	♂	22	6.5.52	1650	57		2.4	Psychosomatic syndrome.	175
22	♂	23	28.1.52	600	47		7.8	Pheochromocytoma. ?	150
	♂	24	23.1.52	756	34		4.5	Unknown.	
			24.1.52	800	48		6		
			25.1.52	1500	52		3.5		
22	♀	25	30.4.52	1650	39		2.4	Hypertension. Tetania ?	160
24	♂	26	6.5.523	60	27	65	4.3	Neurosis	130

e history. Patient No. 1 was a 24 ld female who, for the period of 10 preceding admission, had suffered sud- ttacks of diaphoresis, headache, sensa- of pressure in thorax, palpitation, tre- nd polydypsia, often after stress or The systolic blood pressure changed a few hours from 180 to 305 mm Hg ie diastolic from 115 to 140 mm Hg ulse rate from 90 to 125 per minute. findings were increased fasting blood (149—168 mg per cent), glycosuria uresis (an average of 1720 ml). After ack there was a feeling of weakness the following day. No tumour was ed or observed radiographically. The showed enlargement by roentgeno- the ECG revealed lowering of the ST nt in all leads. The patient was ed on the right side; no tumour was ed. The patient left the operating in good condition, but expired sud- 15 minutes later. The pathological nation revealed a paraganglioma of e of hen's egg close to the aorta and ventrally. The tumour weighed

he second patient the mean excretion adrenaline was 354 μg per 24 hours d from 47 to 590 μg) and the con- ion 33 μg per cent (ranged from 68 μg per cent). The patient was a r old female who complained periodi- f palpitation and headache. Attacks een in the hospital with simultaneous n blood pressure and an increase in rate. One month later the excretion rmal.

ie third patient the excretion of nor- line ranged from 80 to 1390 μg per day, the highest value being observed during one day only. During 5 other days the excretion was less than 230 μg per day. The concentration was 15,5 μg per cent (ranged from 3 to 58 μg per cent). Since the excretion of noradrenaline was normal in these later tests and the patient had been severely injured in the war, operation was not indicated.

In two other patients (No. 4 and No. 5) the excretion of noradrenaline in the urine was normal. These patients had hypertension and the radiological examinations revealed ren duplex (No. 4) and ureter and pelvis duplex (No. 5). Also phaeochromocytoma was suspected. In laparotomy no adrenal tumour was found. Patient No. 6, who had a normal excretion of noradrenaline, but other symptoms suggestive of phaeochromocytoma (hypertension), was also operated on the other indications. The excretion varied for 5 days from 36 to 145 μg per day. The concentration ranged from 3 to 16 μg per cent. An aneurysm of the abdominal aorta was found.

In 20 other patients the excretion of noradrenaline was also within normal limits or slightly increased. Most of these patients belong to the group of hypertensives and a few to the group of neurotics; one had metastasizing carcinoma from the stomach, and one had hyperinsulinism. In 9 patients (Nos. 7 to 15) with high systolic (200—290 mm Hg) and high diastolic (17 had over 140 mm Hg) blood pressure, the average excretion was 94 μg per day. In 11 other patients the excretion has been 71 μg per day. (Nos. 16 to 26 with systolic blood pressure from 130 to 170 mm Hg and the diastolic from 70 to 100 mm Hg.)

Table III. *Excretion of noradrenaline and adrenaline (as noradrenaline equivale in the urine after intravenous infusion of noradrenaline in patients with surgical shock.*

		1. day			2. day			3. day		
Patient No.	Noradrenaline infused μg	Total volume of urine ml per 24 hrs.	Excretion μg	Concentration μg per cent	Total volume of urine ml per 24 hrs.	Excretion μg	Concentration μg per cent	Total volume of urine ml per 24 hrs.	Excretion μg	Concentration
1.	5000	1290	410	32	650	205	30	1490	400	
2.	8000	1000	420	42	2165	190	24	665	191	
3.	5000	600	500	83	600	319	53	750	196	
4.	10000	800	281	35	800	200	25	450	247	
5.	5000—10000	950	214	23	500	90	18	590	108	
6.	15000	350	88	25	450	61	14	650	94	
	average	**1023**	**319**	**40**	**861**	**178**	**27**	**766**	**206**	

To determine what part of adrenaline and noradrenaline appears in the urine in conjugated form, the determinations have been made in some cases after acid hydrolysis in pH 1.5 during 30 min. This procedure hydrolyses the sulphates. In patient No. 1 with paraganglioma, large amount of noradrenaline (average 48 per cent) has been found in conjugated form. In 5 other patients a relatively small amount has been in conjugated form (24 per cent).

Excretion in surgical patients after an intravenous infusion of noradrenaline.

(Table III)

We also determined the excretion of noradrenaline in 6 surgical patients who were given noradrenaline as an intravenous infusion in the treatment of surgical shock. The total dosage of noradrenaline per patient varied from 2500 to 15 000 μg. Collection of urine specimens was started at the beginning of infusion and continued for 3 The mean excretion of noradrenaline fo first day was 319 μg, during the second 178 μg and during the third day 20 and the mean concentrations in the respectively, 40 μg per cent, 27 μg per and 28 μg per cent.

DISCUSSION

The normal excretion of noradrenali large enough to be determined in the and therefore gives a better picture of pathetic nervous system activity tha determination from the blood. In the from normal urine the total fluores reaction of noradrenaline and adrenali easily observed after manganese di oxidation and the addition of alkali ascorbic acid, even when diluted 20 In the eluate from normal urine the flu cence reaction produced after additi alkali without manganese dioxide oxi

tively weak, because the amount of enaline in normal urine is much than the amount of adrenaline. The ntiation of noradrenaline from adre- is easier in cases of increased excre- ecause in such cases, as in phaeochro- oma, the fluorescence reaction due to radrenaline or adrenaline is often 20 times stronger than in normal urine. mean concentration of noradrenaline drenaline determined by this specific cence method in normal urine is con- ly higher (3—17 μg per cent) than normal peripheral blood (0.3 μg per f noradrenaline and 0.1 μg per cent enaline). During increased excretion e urine of the patient with paragang- there was a mean concentration of per cent with a maximum concentra- 150 μg per cent. The concentration drenaline and adrenaline in the blood ients with phaeochromocytoma has nly 1.4 to 9.8 μg per cent (Lund & , 1951, Lund, 1952.)

values for normal urine have been hat higher than figures obtained by cal methods (v. Euler & Hellner, 1952). When determined as the nor- line equivalent, we can assume that lues are somewhat higher than if nor- line and adrenaline had been deter- separately. The normal excretion has udied in students during daily activity n two instances, after examinations. excretion of noradrenaline and adre- into the urine is representative of rt which has not been metabolized in ganism and, therefore gives only a picture of the total activity of adrenal- hetic nervous function. The metabolic capacity of the organism is an important factor which influences the noradrenaline and adrenaline content of the urine. Goldenberg and Rapport (1951) who used biological methods, have stated that the noradrenaline in urine is chiefly due to secretion from the peripheral sympathetic nerve endings and, to a lesser extent, due to secretion by the adrenal medulla.

It is important to know what proportion of infused noradrenaline and adrenaline can be recovered in the urine. The amount of noradrenaline after infusion was considerably higher in the urine than would be expected normally in the absence of shock. But only under 10 per cent of the total amount infused intravenously has been observed in the urine, the largest part having been metabolized in the organism. To some extent increased values in urine are also due to the increased endogenous secretion of adrenaline and noradrenaline in shock from the adrenal medulla and sympathetic nerve endings (Franksson, Gemzell & v. Euler, 1954). By using biological methods also only 1—3 per cent of the infused noradrenaline was found in the urine (v. Euler & Luft, 1951).

In shock the ability of the kidneys to excrete adrenaline and noradrenaline has possibly been disturbed. In one case (No. 6) of severe shock the excretion of noradrenaline was only normal and recovery in the urine very low in spite of the infusion of 15 000 μg of noradrenaline. In this case the amount of urine has been very small. According to Werkö, Bucht, Josephson & Ek (1951), noradrenaline, although producing renal ischemia when administered in large doses, does not affect the secretion of urine. It is interesting to recall, in this connection, the

observation of v. Euler and Luft (1949) that the adrenocorticotrophic hormone will reduce the excretion of noradrenaline in the urine; in shock the effect of increased excretion of endogenous adrenocorticotrophic hormone must be taken into consideration (Uotila & Pekkarinen, 1951). In cases of less severe shock the recovery of noradrenaline in the urine was relatively large despite the infusion of small amount of noradrenaline.

On the other hand, evidently, in phaeochromocytoma or paraganglioma (patient No. 1), the organism is capable of eliminating the excessive amount of noradrenaline and adrenaline in the urine, possibly as a compensatory adaptation mechanism, and in relative large proportion in conjugated form.

SUMMARY

The normal mean daily excretion of adrenaline and noradrenaline in the urine in 17 medical students has been 81 μg and the concentration 8.7 μg per cent during daily activity. In 26 patients with hypertension and some other pathological conditions one had a paraganglioma with highly increased excretion of noradrenaline in the urine, an average of 1354 μg per day and the concentration on the average 80 μg per cent. In two other patients increased excretion has also been found which was later normalized. In 23 other patients increased excretion has not been found when compared to the values of normal individuals.—In patients receiving infusions of noradrenaline in surgical shock a few per cent of the amount of noradrenaline infused was found in urine during 3 days after the infusion.

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ERLIPEMIA AND HYPERGLYCEMIA IN DIABETES MELLITUS MONSTRATION OF ARTERIOVENOUS LIPOID DIFFERENCE

By P. H. D. WAAGSTEIN

From the Maribo County and City Hospital, Medical Department, Maribo, Denmark

(Received for publication Aug. 13, 1954)

he continuous stream of papers ap- g on the relation between the blood and the blood lipoid values found in s mellitus, American investigators h, Phibbs & Carbonaro, 1953) have d quite recently the existence of lism between these two.

question has been dealt with pre- by Blix (1925), Katch & Krainick , Hataeyama, Takahasi, Tutumi & aki (1939), and a complete biblio- of the older literature on diabetic ipemia has been given by Geelmuyden , Fischer (1903) and Allen (1902). ome degree the question depends on methods have been employed for the inations and which fraction or frac- nave been determined. Hirsch *et al.*, stimated the different fat fractions in od, found in animal experiments that l fat shows the greatest increase, as cholesterol and phospholipids are sed but insignificantly or not at all. he question thus still is under discus- t seemed of interest here to present ndings in some studies carried out in -46 in the Maribo Hospital. The l here employed for fat determina- as the hemolipocrit method given by rt, which is a modification of Gerber's method for milk fat determination (Rückert, 1931; Burger, 1939 and Lawaetz & Vogt Møller, 1934).

The present material comprises 50 diabetics, the age and sex distribution of whom is shown in Table I.

Determinations of the fasting blood sugar and fasting serum lipoid values were performed on all the fifty patients through the entire period of hospitalization.

The total material has been treated statistically. On comparison of the relation between sugar and blood lipoids on admission and discharge of the patients, the regression coefficient is found to be highly significant in either instance. The regression coefficient is greater for diabetics under 40 years ($b = + 1.651$) than for diabetics over 40 years ($b = + 1.211$). As to normal values for total serum lipoid *ad modum* Rückert, see Waagstein (1948).

The outcome of these calculations is presented graphically in Figs. 1 and 2.

On studying the individual curves — which for the sake of space have been left out here — certain peculiar things stand out conspicuously. Even though these deviations counterbalance each other in the summary graphs, they still should be mentioned in particular as they raise some new problems.

Table I. *Age and sex distribution of the present material.*

All the patients entered here were admitted to the Maribo Hospital. Their treatment was the same as usually employed in this country. The patients were kept in bed till the various tests had been made.

Unfortunately, the cases with development of gangrene could not be followed up as they were transferred to the Surgical Department. Some of these patients were, however, readmitted to our clinic after amputation had been performed.

Age	Males	Females	Total
0— 5 years	0	1	1
6—10 »	0	0	0
11—15 »	0	0	0
16—20 »	1	0	1
21—25 »	2	2	4
26—30 »	0	1	1
31—35 »	2	1	3
36—40 »	2	2	4
41—45 »	2	2	4
46—50 »	0	1	1
51—55 »	2	6	8
56—60 »	2	6	8
61—65 »	1	8	9
66—70 »	2	3	5
71—75 »	0	1	1
			50

Among others, this applies to Case and 29, in which the hyperlipemia rose high level and could not be reduced in of normalization of the hyperglycemia. even presented the peculiar feature tha hyperlipemia increased on confineme bed, being thus suggestive of some m nical factor. These two patients showe sign of arteriosclerosis. If their prog was to be made on the basis of the h lipemia (cf. Rabinowitch) it would be — on which account a follow-up exa tion would have been very interestin another patient (Case 6) — with retino — attempts to reduce the hyperlipem intensive treatment likewise failed.

In Case 26 (Fig. 3) a striking observ was made. Here the performance of lu puncture was followed by a sudden ri temperature to 40° C and disappearan the hyperlipemia, so that the serum, v before had been very milky, now be

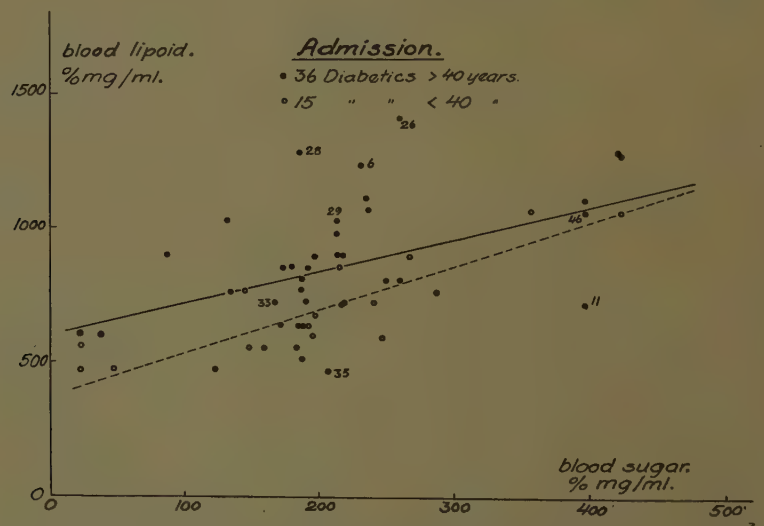


Fig. 1. Relation between the values for blood sugar and blood fat in 36 diabetics over 40 years and 15 under 40 on admission to the hospital.

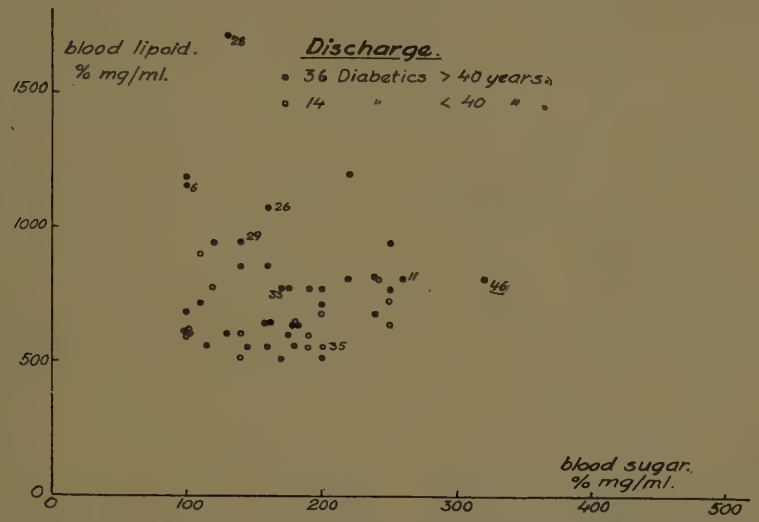


Fig. 2. Relation between the values for blood sugar and blood fat in 336 diabetics over 40 years and 15 under 40 at discharge from the hospital.

Nothing was found that might explain se in temperature, and in the course w days the originally high blood lipoid as restored. This phenomenon — pro- ed effect of the body temperature upon te of hyperlipemia — was observed n a patient who had an attack of onia.

o (1933) states that alimentary hyper- n is lowered on rise in the body tempe- So far, however, we have not been find any information in the literature he influence of the body temperature diabetic hyperlipemia. Perhaps this nenon explains the observation re- by Joslin concerning acidosis without pemia — something we have never ed. Presumably it must have been the s produced by the fever that has not ccompanied by hyperlipemia.

Conversely, in Case 35 the striking feature was the absence of hyperlipemia, although the state of decompensated diabetes was suggestive of active pancreatitis. The diagnosis of pancreatitis is very difficult (cf. Prati (1942) and La Barre, Katch *et al.* (1939). Some French investigators — *e. g.*, Mauriac, Servantie & Baron (1941) — think it is possible by fat tolerance experiments to disclose the presence of pancreatitis.

All of our patients that were admitted in a state of insulin chock (see Table II) showed a very low level of total blood lipoid. This observation is in agreement with findings reported by other authors (*e. g.*, Katch, Bertram and others, cited after Fischer) and confirmed through more recent animal experimental observations (cited after Katch & Krainick). Likewise, it is in keeping with the more recent observations reported by

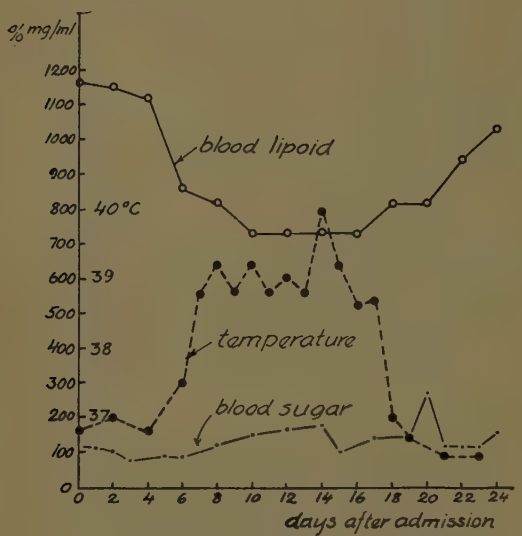


Fig. 3. Influence of the body temperaure on the course of fasting lipemia in diabetics.

Table II. *Blood sugar and total blood lipoid value in patients admitted in insulin shock.*

Record No.	Sex	Age (years)	Blood sugar	Total blood lipoid
275/45	M.	43	30 mg.	430 mg.
755/44	M.	35	65 »	473 »
573/45	F.	21	30 »	473 »
	M.			602 »

Rickets, Brunschwig & Knowlton (1946). According to these authors, patients on whom total pancreatectomy had been performed showed increased sensitivity to insulin and frequent occurrence of insulin shock. In these patients the fat absorption has been defective, and hence the blood fat level must be assumed to have been low.

Altogether 12 of the present patients entered the hospital in a state of coma, and in all these cases the hyperlipemia had reached a high level (Table III). After regulation of the diabetes these values returned

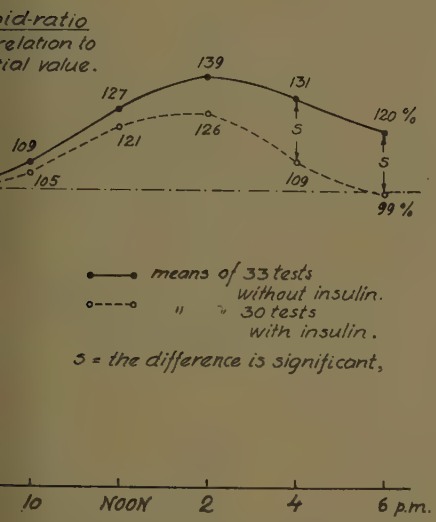
Table III. *Blood sugar and total b lipoid in mg % in patients admitted state of coma.*

Record No.	Sex	Age (years)	Blood sugar	Tot blood l
93/46	M.	24	360 mg. %	1075 m
588/45	M.	23	400 »	1075
1215/45	F.	52		1075
459/42	F.	54		1118
42/45	F.	63	400 »	1075
600/45	F.	63		1935
81/45	M.	56		1290
305/45	F.	14		1290
				1161
				1075
1494/46	M.			1935
1428/46	M.			2700

to a normal level, and in no case — as st by various authors (*e. g.*, Blix) — acidosis observed without hyperlipemi

In addition, fat tolerance tests were ried out on 30 selected patients. The fa patients were given 100 g peanut oil, determination of the blood fat perfo every other hour for 10 hours. On days the patients were kept fasting and merely a meal of vegetables without a at noon. After a pause of 1 day the fat rance test was repeated simultaneously administration of insulin, which had discontinued the first test. In contra Blix, the course of the alimentary curv found to be flattened when insulin was simultaneously with the oil (see the sum curve in Fig. 4).

The appearance of the hyperlipemic however, will depend on the stage course of treatment when the tolerand is performed. The earlier the test is formed and the more poorly the di condition of the patient has been regu the higher is the peak of the curve.



Course of alimentary hyperlipemia in 30 after intake of 100 g peanut oil. In the t no insulin was given; in the second test ch patient received 40 I. U. insulin.

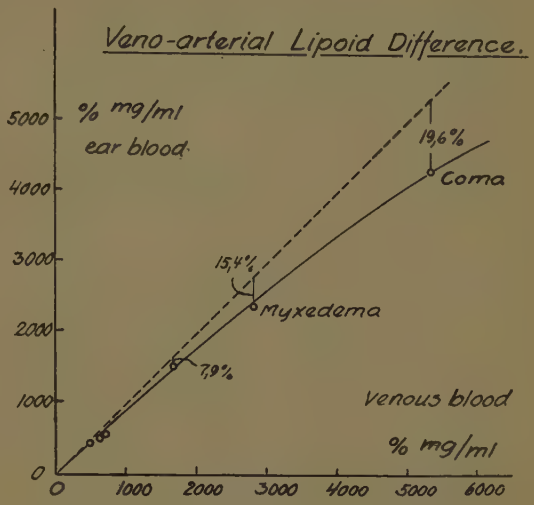


Fig. 5. Difference in the level of lipemia in serum from ear blood and in serum from venous blood.

insulin effect here ascertained, how- ay also have been indirect. For, even the patients were fasting, it cannot out of account that in the first fat ce test the diabetic state of the patient ve been aggravated by the omission lin.

ost studies on diabetic hyperlipemia en carried out by means of cholesterol ination, we have also performed some ative examinations with determination cholesterol content on venous blood. method employed for determination cholesterol content of the blood re- venepuncture. At the same time, was obtained from the venous blood determination according to Rückert. e analyses constantly revealed a dis- fference in the lipemia depending on r the serum came from blood obtained by venepuncture or by a stab in the ear (see Fig. 5). Recently this phenomenon has been observed in animal experiments by Törnblom (1953) who withdrew the two kinds of blood samples simultaneously from an artery and a vein, respectively. Whether this phenomenon is to be explained physically as owing to differing oxygen tension in venous blood and in arterial blood is a question that requires further experimental study.

In animal experiments, French investigators (Roger & Binet) have found a difference in the lipoid content of venous blood withdrawn from the right ventricle of the heart and of arterial blood. These investigators have advanced the theory that the lungs might play a role in fat metabolism corresponding to that of the liver in carbo-

hydrate metabolism. The question then arises: Why does the fat pass outside the portal vein over into venous blood and through the lungs before it enters the systemic circulation?

And this question is still unsettled.

SUMMARY

Fifty cases of hospitalized diabetics have been followed with determination of the total lipoid content of the blood besides determination of the fasting blood sugar. The two curves are found to run parallel.

Two cases of pronounced hyperlipemia showed a course quite independent of that of hyperglycemia. The rise in hyperlipemia on confinement to bed appears suggestive of mechanical factors.

Fever is found to lower the level of fasting lipemia.

Thirty fat tolerance tests with and without administration of insulin illustrate the influence of insulin upon the course of alimentary hyperlipemia.

Accidentally a constant arteriovenous lipoid difference is ascertained on determination of the total lipoid content on blood obtained from the ear and on venous blood.

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AN EXAMINATION OF THE RELATION BETWEEN ERYTHROCYTE-AUTO-ANTIBODIES AND SYPHILITIC REAGI

By V. AHRENGOT and HENNING SCHMIDT

From Statens Seruminstitut, Copenhagen, Denmark

(Received for publication Aug. 20, 1954)

The purpose of this study was to examine whether a relation exists between the reagins demonstrated by syphilitic seroreactions and the so-called erythrocyte-auto-antibodies (E-a-a).

E-a-a are responsible for the hemolysis in certain hemolytic anemias. They are globulins with affinity to erythrocytes, but ordinarily they are hardly generated by imunization with erythrocytes. Nothing definite is known as to their origin, but many indications suggest that their presence is due to infections, *e. g.* the cold agglutinins occurring in atypical pneumonias (Finland, 1945; Siim, 1946). However, no detailed explanation can be given of the relations between the three factors: the infecting agent, antibodies and erythrocytes (Ahrengot, 1954).

The reagins demonstrable by syphilitic seroreactions are globulins with affinity to lipoproteins. As the reagins do not always arise as a result of an infection with *Treponema pallidum* they can hardly always be specific spirochetal antibodies. This will be seen, for instance, from the fact that positive seroreactions can sometimes be demonstrated in persons who never had a syphilitic infection or any other spirochetal disease. In this country such biologically false positive reactions occur especial patients with infections of the respir tract (Krag & Lønberg, 1938; Wiing 1948; Schmidt, 1953); in pregnant w biologically false positive seroreaction occur more frequently than in clin healthy non-pregnant persons (Sch 1954). Finally, biological false positiv actions occur in completely healthy p in about 0.2 per cent of the examined viz. 0.19 per cent in men and 0.31 pe in women (Schmidt, 1954).

Donath & Landsteiner (1904) d strated E-a-a (hemolyzing antibodies) serum of certain syphilitics and later & Lager (1951) were able to demon that the frequency of cold-agglutinins from syphilitics was greater than th quency of cold-agglutinins in sera a control group consisting of patient fering from diseases not ordinarily con with elevated cold-agglutinin titre.

An examination of a group of p with E-a-a revealed that some of the positive syphilis reactions without clini anamnestic data to suggest syphilis. the patients had been given anti-syp treatment for a long time without sh any drop in the seroreactions. In this

ochetal immobilization test T.P.I.$_{50-18}$ n & Meyer, 1949) was negative, in- ; that the seroreactions were probably n-syphilitic nature.

MATERIAL

samples examined fall in five groups: 292 blood samples which showed posi- ults in one or more seroreactions for (see below) were examined for These samples were taken at random the 40 days between June 12th and 0th, 1953 (with about seven samples among the positive sera found by the iagnostic Department in that period. ard was taken to the etiology of the ctions in this group.

140 blood samples submitted to the iagnostic Department between Janu- st and May 18th, 1953 and showing e results in one or more seroreactions hilis were examined for contents of The seroreactions indicated that amples were non-specific, showing a ancy in the seroreactions.

159 blood samples serologically exam- r syphilis between June 9the and June 953 but showing negative results in incipal tests« C-WR-M and KR (see , were examined for E-a-a.

116 patients (*viz.* all patients regi- in the Blood-Grouping Department, sitive E-a-a-reactions which were not Rhesus incompatibility) showing or previously shown positive reactions a-a were examined in the Central r of Syphilitic Patients (Jersild, 1919; n & Krag, 1937, Schmidt, 1951 and 1954).

blood samples from patients of this were received from medical hospital departments. As medical departments in Denmark submit blood samples for examination for syphilis from nearly all patients, those showing positive syphilitic reactions (regardless of etiology) or who have clinically diagnosed syphilis (even if the seroreactions are negative) will be found in the Central Register of Syphilitic Patients.

(e) 102 patients whose blood samples in examinations for E-a-a showed negative results, were examined in the Central Register of Syphilitic Patients. These patients were selected by taking every 20th patient among those whose blood — under suspicion of hemolytic anemia or related diseases — had for a certain period been examined for E-a-a with negative results.

METHODS

The examinations for E-a-a were carried out by:

(1) Coombs' anti-globulin reaction in the direct form (Coombs, 1946). The principle of this examination is that erythrocytes affected by antibodies will agglutinate in rabbit immune serum containing antihuman-gamma-globulin, because the antibodies consist of gamma-globulins. Normal erythrocytes do not agglutinate.

The results have been described as "positive", "doubtful positive" and "negative" (valued according to the standard practice adopted by the Blood Grouping Department).

Supplementary examinations were carried out with:

(2) Suspension of the erythrocytes in "Macrodex", a very agglutinogenic medium which may sometimes cause erythrocytes affected by antibodies to agglutinate, while normal erythrocytes do not agglutinate (Eldon, 1952; Ahrengot, 1954).

The following seroreactions were used for examination of the syphilitic reagins:

(a) Wassermann's Complement Fixation Test as modified by Mørch with cardiolipin antigen, C-WR-M (Schmidt, 1951, 1952; Schmidt & Lundbäck, 1954).

(b) Kahn's Standard Reaction, KR (Kahn, 1928).

The principal reactions, C-WR-M and KR, are routine examinations for all sera received, but if the result of a preliminary qualitative examination is not completely negative with one or both antigens, the reagin content of the serum is determined quantitatively in C-WR-M and KR and examined, at the same time, in

(c) Meinicke's Clarification Test, MR, (Meinicke, 1932). This test in only partially quantitative, the results being described as "strong positive", "weak positive", "doubtful positive" and "negative".

AUTHORS' EXAMINATIONS

Table I shows that 4.4 per cent of all the samples with seropositive reactions for syphilis, regardless of etiology, show definitely positive E-a-a reactions. None of the 159 samples which were negative in C-WR-M and/or KR show definitely positive E-a-a reactions. Of the sero-positive reactions for syphilis 5.5 per cent show doubtful positive E-a-a reactions, against 0.6 per cent of the sero-negative reactions for syphilis.

Table II shows that 11.2 per cent of the 116 E-a-a positive patients had positive seroreactions for syphilis, against only 2 per cent of the 102 E-a-a-negative patients. The sex and age distributions of the groups (d) and (e) were practically alike.

In all the above comparisons of relative incidences the differences are statistically significant (the so-called P-value (Hald, 1952) being less than 5 per cent in all cases).

In order to ascertain whether the syphilitic infection is responsible for the positive E-a-a reaction when syphilitic reagins and E-a-a occur together, or whether — as we believed when the study was started — only the diseases causing biologically false positive seroreactions are responsible for the coin-

Table I. *Syphilis sero-positive (A) sero-negative (B) samples examined for tents of erythrocyte-auto-antibodies (E-*

		E-a-a-reaction positive	dou
A. Group (a):	292 sera	12 = 4.1 %	18 =
Group (b):	140 sera	7 = 5.0 »	6 =
Total	.....	19 = 4.4 »	24 =
B. Group (c):	159 sera	0	1 =

Table II. *Number of E-a-a-exam patients (group (d): positive E-a-a; (e): negative E-a-a) registered in the C Register of Syphilitic Patients.*

Group (d):	116 sera	13 = 11.2 %
Group (e):	102 sera	2 = 2.0 »

cidence, the syphilis sero-positive sa have been divided into three groups (III), *viz.* patients with (1) syphilis (cl and/or 'anamnestic), (2) patients wi clinical or anamnestic indications of sy where the seroreactions became spon ously negative or where the T.P.I was negative, and (3) patients for w the available data did not enable u prove or disprove the diagnosis of syp A large part of Group (3) consis patients who showed strong positive reactions for syphilis, presumably of s litic etiology, even though no clinic anamnestic data on syphilis were aval However, as the purpose was to eva serological data, it would not be justifia apply serological criteria to a diag evaluation.

e III shows that both positive and il positive E-a-a-reactions are distrib- irly evenly over the three groups and istically significant differences could ertained between the numbers of neously positive reactions found in ee groups. Hence, it will be justifiable clude that a coincidence of positive reactions and positive seroreactions for s has no relation to the etiology of the ns but is an indication of an intimate ical affinity.

lly, the patients with sero-positive th sero-negative reactions for syphilis een classified by age and sex in order ertain whether any particular group be particularly heavily represented, s was not the case.

diagnoses reported for the non-syphi- tients with positive seroreactions for s were very dissimilar and did not any classification of the patients in ly separate groups; in several cases gnoses could hardly have any relation positive seroreactions.

ONCLUSION AND DISCUSSION

findings reported in the present paper strate that simultaneous presence of ic reagins and erythrocyte-auto-anti- (E-a-a) is more frequent than can be ed by coincidence. It has also been strated that simultaneous presence of o groups of antibodies may occur not n syphilis but just as well in non- ic diseases. Thus, the correlation e assumed to be purely serological and ndicate a close relationship between and the syphilitic reagins.

Table III. *432 seropositive samples, Groups a & b.*

A: Samples from syphilitics.
B: Samples from non-syphilitics.
C: Samples from patients with unstated diagnoses.

	Erythrocyte-auto-antibody reactions	
	positive	doubtful
A. 207 sera	9 = 3.4 %	12 = 5.8 %
B. 175 sera	9 = 5.2 »	9 = 5.2 »
C. 50 sera	0	4 = 8.0 »

This serological affinity leads to speculations about other points of similarity — etiological and clinical — between the two groups of antibodies.

"Syphilitic" reagins are known to occur simultaneously with infections, although no details are known about the mechanism that is responsible for the presence of the syphilitic reagins. As stated previously, the etiology of E-a-a has not been fully cleared up, but an application of the experience with syphilitic reagins to E-a-a lends support to the theory that E-a-a is caused by infections. (Statts & Wassermann, 1952).

A study of the syphilitic reagins in the light of our knowledge of E-a-a poses several questions. E-a-a are known to have an affinity to all human blood corpuscles, but except for their non-specificity they behave serologically exactly like blood-group antibodies. As their optimal temperature is known usually to be lower than that of the human body, they can hardly owe their existence to immunization with the erythrocytes of the human body (the optimal temperature of all known immune blood-group antibodies being that of the human body).

There is nothing to suggest that the erythrocytes play any part in the formation of E-a-a; nor is there actually any proof that the E-a-a are the result of an immunization process. E-a-a may just as well be the result of the direct effect of the infectant on the serum proteins, their agglutinin character thus being comparable to the effect of the agglutinins found for instance in the juices of numerous plants.

Concerning the syphilitic reagins, etiological considerations very much like those made for E-a-a may be applied. It is known that the existence of syphilitic reagins is related to an infection, but nothing is known as to the character of that relation. Whether lipoproteins like those used for the demonstration of the reagins play any rôle in their generation is quite uncertain.

Even if E-a-a are not erythrocyte immune-antibodies, there can be no doubt of their effect on the red blood cells. Both *in vitro* and *in vivo* they cause evident damage to the erythrocytes; in patients this is manifest as hemolytic anaemia.

So far, no studies appear to have been made of the importance of syphilitic reagins to the pathogenesis of syphilis. In the light of the above observations concerning E-a-a it would seem to be quite probable that syphilitic reagins are not only of diagnostic importance but that they may be responsible for some of the conditions previously ascribed to *Treponema pallidum*. In the same way as E-a-a affect the erythrocytes, the reagins might conceivably cause damage to the tissue containing those lipoproteins to which reagins react as a kind of antibody.

SUMMARY

A study on the relationship bet erythrocyte-auto-antibodies (E-a-a) and s ilitic reagins is presented.

The examination reveals a correlatio twen the two sorts of antibodies. The co tion is found in patients with syphilis a as in patients with biologically false pos seroreactions. Thus the correlation is preted as a serological rather than an logical affinity.

The consequences of the relationship monstrated are discussed, and in analogy the well-known effects of E-a-a upon th corpuscles the hypothesis is proposed syphilitic reagins may have clinical e and possibly be responsible for some syphilitic manifestations.

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THE EFFECT OF FREE FATTY ACIDS ON THE MIGRATIO RATES OF LIPOPROTEINS IN PAPER ELECTROPHORESIS

S. LAURELL

From Laboratory of Clinical Chemistry, University of Lund, Lund, Sweden

(Received for publication August 21, 1954)

In 1952 Rosenberg showed that the migration rates of human α_1- and β-lipoproteins in paper electrophoresis increased 35 minutes after the injection of heparin in subjects with alimentary hyperlipemia. This was confirmed by Lever, Smith & Hurley (1954), who observed a similar phenomenon during experiments with moving boundary electrophoresis. These authors suggested that the changes in migration velocities might be directly caused by heparin. However, the concentration of heparin needed *in vitro* to reproduce the electrophoretical changes has been found to be much greater than the concentration presumed to occur in the human circulation after intravenous injection (Hoch & Chanutin, 1952, Nikkilä, 1953). For this reason the above explanation can hardly be sufficient.

In the experimental part below it will be shown that the phenomenon observed by Rosenberg (1952) can be produced in *in vitro* experiments by the aid of a "clearing system". Since it was shown by Shore, Nichols & Freeman (1953) that free fatty acids are liberated during the "clearing process", it seemed to be of interest to investigate if addition of free fatty acids to serum influence the migration velocities of lipoproteins. It has been shown by Armstrong, Budka & Morrison (1947) that the mobilities and β-lipoproteins increase in m boundary electrophoresis when the s salt of caprylic acid is added to the d barbituric acid buffer. Some experi with addition of pure albumin to serum included with regard to the known ca of albumin to bind fatty acids. (Revi this literature see Murray Luck, 194

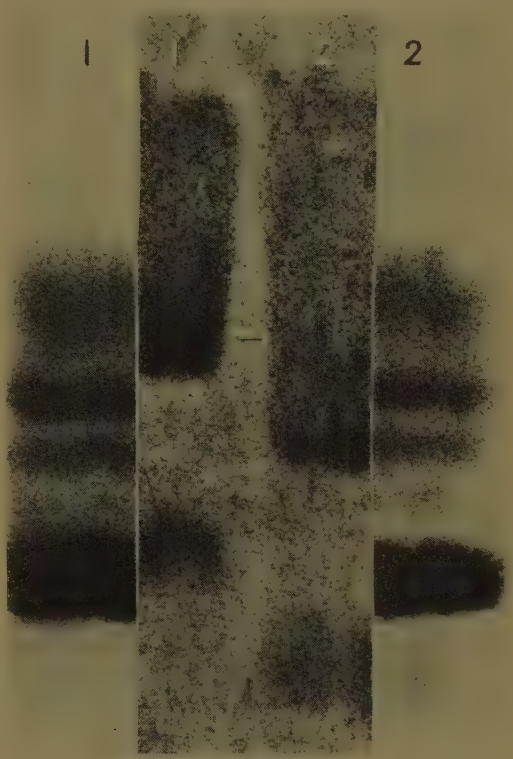
METHODS

The paper electrophoresis was performe moist chamber similar to that described by Svensson (1954). Paper strips (78 × 350 m Whatman No. 1 were used. 20 cmm of tw were applied to each paper as two 20 mm lon Two such papers could be placed side by the same apparatus. The buffer (pH 8.6) de by Michaelis (1931) was used. The electrop was run for 12 hours at 120 V. γ-globulin m 20 to 25 mm in anodal direction presumably evaporation. The papers were cut before in one central and two lateral parts to e the relative positions of proteins and lipop The lateral ones were stained for protei bromphenol blue, the central one was stai lipids according to Swhan (1953). Sometim whole papers were colored first for lipids a for proteins. Invariably the results were th with both methods.

Pooled fresh serum from blood donors a diers was used. About 1.7 mg of the disodi of ethylenediaminetetra-acetic acid (EDT was added per ml of serum to prevent preci

m and magnesium soaps in the experi-
nen oleic acid was added. EDTA-Na_2 in
entration had no apparent effect on the
oretic results. Artificial lipemic serum
uced from pooled serum by addition of
per cent of chylous ascitic fluid (total
per cent determined with the method of
1953)). This fluid was obtained from a
ith obstruction of the thoracic duct.
ing factor" was purified according to
(1953) from serum of medical students.
um was obtained from blood withdrawn
after the injection of 50 mg of heparin.
ipitated clearing factor was dissolved in
osphate buffer (pH 7.75) to the original
In order to obtain clearing, 1—2 volumes
lution were added to 10 volumes of lipemic

cid was mixed with serum in the following
Oleic acid (Kebo, analytical reagent)
d to flat bottomed test tubes by the aid of
micrometer syringe" in amounts of 0.4
m per ml of serum. The serum, as a rule
as added afterwards and mixed with a
stirrer. Control experiments showed that
of 3 cmm of oleic per ml of serum caused
e in pH from about 7.8 to 7.3. Compensa-
his by addition of N NaOH was without
the electrophoretic results and therefore
cluded.
uman albumin (97—98 per cent pure) was
pplied by AB. KABI in freeze-dried state.



Paper electhrophoretic patterns of serum before (1) and after (2) addition of 3 cmm of oleic acid per ml. The central part is stained for lipids and the lateral ones are stained for proteins.

RESULTS

r prevailing experimental conditions
t of the lipid-stainable fraction corre-
g to β-lipoprotein was found between
and β-proteins. The Sudan black-
e α_1-lipoprotein was found, in accord-
th Swahn (1953), in the region of
, overlapping the zone between this
protein.
"clearing" for 20—30 hours at room
ture with clearing factor, the β-lipo-
front coincided with that of α_2-pro-
most of the α_1-lipoprotein was found in front of albumin. A new bromphenol blue stainable zone was also found in this region and the faintly stained zone between albumin and α_1-protein had decreased in color. Control sera containing buffer instead of clearing factor solution showed no significant change in the position of lipoproteins after 20—30 hours' incubation, when compared with their regular sites, nor did lipemic serum with added clearing factor if submitted to electrophoresis without preliminary incubation.

The effect of the clearing reaction on the migration rates of the lipoproteins was re-

versed by addition of 50—100 mg of albumin per ml of serum.

After addition of oleic acid to serum no change in the migration velocites of the different lipoprotein fractions was observed, if the added quantity was below approximatively 1 cmm (0.9 mg) per ml of serum. The migration rates of α_1- and β-lipoproteins increased with increasing amounts of added oleic acid in the region from 1 to 2.5 cmm per ml of serum. Addition of further amounts of oleic acid was without influence. The maximal changes in the migration velocities of the lipoproteins promoted by addition of oleic acid to serum corresponded to the changes obtained in the experiments with the clearing system. The effect of oleic acid was reversed by albumin.

In some of the experiments with addition of oleic acid, a spot was observed which was faintly colored by Sudan black, behind the α_1-lipoprotein in the region of albumin. However, it had more bluish tint than the other stainable matter. So far its nature has not been elucidated. A solution of human albumin (5 per cent in 0.9 per cent NaCl) only gave a scarcely discernible spot, when 20 cmm was subjected to electrophoresis and colored with Sudan black. This spot was not visibly intensified by addition of 2.5 cmm of oleic acid per ml.

DISCUSSION

The observed influence of oleic acid on the migration rates of the lipoproteins may be due to a blocking of positively charged groups on their surfaces or to an adsorption of the fatty acid to these proteins leaving its carboxylic group partly dissociated. The capacity of albumin to reverse the electrophoretic changes produced by oleic aci the lack of effect by this acid when le 1 cmm of it had been added per ml of s may be explained if albumin binds the acid more tighly than the lipoproteins it is assumed that this is the case and th concentration of albumin in the pooled is 4.5 per cent, 1 cmm (0.9 mg) of olei (M = 282) per ml of serum would spond to about 5 moles of oleic acid per of albumin. This value corresponds lower figures reported in the lite (Murray Luck, 1949). Of course som fatty acids may have been present, bou albumin, already before the addition o acid.

It has been shown by Robinson & F (1953) that albumin is a limiting fact the clearing reaction of lipemic rat p *in vitro*. This was interpreted to be d the saturation of albumin with fatty As the lipoprotein concentration o plasma is far below that of human s it is possible that clearing may proce human serum even after the albumi been saturated, the lipoproteins acti acceptors for free fatty acids. The el phoretic study of a clearing system dese above suggests that this may be the Further investigation of this possibil in progress.

SUMMARY

The clearing of artificial human li serum *in vitro* has been shown to in the migration velocities of the lipopro paper electrophoresis.

The same effect may be produced by tion of oleic acid to serum.

effects may be reversed by albumin. ggested that the free fatty acids liber- ıring the clearing of lipemic serum he observed electrophoretic changes.

ACKNOWLEDGMENT

ıanks are due to AB. Hässle, Hässleholm, .boratory was placed at my disposal during this investigation.

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SPECTROPHOTOMETRIC DETERMINATION OF CREATINI IN ALCOHOLIC SOLUTION

By L. JACOBSSON and L. PAULSEN

From the Central Laboratory, Sahlgren's Hospital, Gothenburg, Sweden

(Received for publication August 30, 1954)

Many methods have been described for the determination of creatinine. Most of them are based on the Jaffe reaction (*e g.* Folin 1904) with numerous modifications, including the use of adsorbents *(e. g.* Borsook 1935) and bacterial decomposition (Miller & Dubos 1937) in order to increase the specificity of the reaction with picric acid.

In the course of time other methods have been introduced and some of them should be mentioned:

With dinitrobenzoic acid, creatinine gives a strong color (Langley & Evans 1936). A nephelometric method has been suggested, using Nessler's reagent as a precipitant (Barrett 1936) but experience with this method is sparse. When oxidizing creatinine to methyl guanidine, the latter can be determined by help of the Sakaguchi reaction (Riegert 1939). Recently some newer principles have been introduced, based on the fact that certain mercury compounds and creatinine form insoluble complexes in which mercury is determined by dithizone (Stelgens, Wolf & Schreier 1950) or creatinine as methyl imide. (Franzen & Göden 1950).

In the present paper a spectrophotometric method for the determination of creatinine in alcoholic solution is described.

EXPERIMENTAL

Creatinine was dissolved in various vents and the absorption in the ultrav region was recorded in a Beckman DU trophotometer, light path 1 cm. The lowing solvents were used: Distilled w methanol, ethanol, propanol, butanol, hep pyridine and hydrochloric acid 0.1 n

In the case of ethanol some further ex ments were performed with varying co trations of water; from 20 per cent t per cent.

A standard curve was obtained at λ mμ with creatinine concentrations from per cent to 2.0 per cent and ethanol-w (4 + 1) as solvent.

In addition, similar experiments were formed with *creatine* in ethanol-w (4 + 1).

The solvents without creatinine or cre were used as blanks.

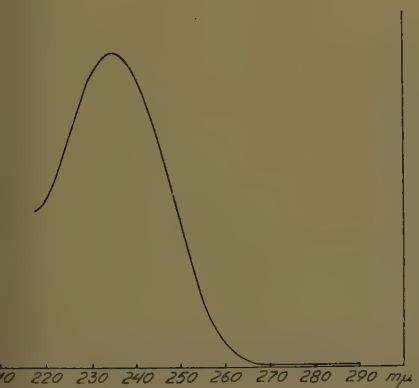
RESULTS

As seen from Table I, creatinine diss in methanolwater (4 + 1), ethanol-w (4+ 1) and butanol shows absorption i ultra violet region with a distinct maxi at 235—236 mμ. With propanol-w (4 + 1), heptane, heptane-butanol (4 and 0.1 n HCl in water no maximum

Table I.

Solvent	Wave-length for max. absorption	Extinction light path 1 cm
1. Methanol-water 4 + 1	235—236	0.620
2. Ethanol-water 4 + 1	235—236	0.725
3. Propanol-water 4 + 1	no max. obtained	—
4. Butanol pure	235—236	0.660
5. Distilled water	no absorption	—
6. Heptane pure	no max. obtained	—
7. Pyridine pure	no absorption	—
8. Heptane-butanol 4 + 1	no max. obtained	—
9. Hydrochloric acid 0.1 n	no max. obtained	—

. Creatinine dissolved in distilled
ıd in pyridine showed no absorption.
g. 1, the absorption curve for crea-
mg per cent in ethanol-water(4+1)
ı. As seen, there is a rather distinct
235—236 mμ. The curve decreases
on both sides of the maximum, re-
he zero level at 270 mμ. At wave
below 217.5 mμ, the absorption of
ık was so high that no light was
ted.



he absorption of creatinine 2 mg per cent
l-water (4 + 1). Beckman DU, light path
1 cm. Solvent as blank.

Experiments with creatinine (4 mg per cent) dissolved in ethanol-water in varying proportions are presented in Table II. With decreasing concentration of water the molar absorption index increases, but it is noteworthy that the difference between the absorptions at the lowest concentrations of water (40 and 20 per cent respectively) is minimal.

Table II.

Ethanol Per cent	Water Per cent	Molar absorbency index 235 mμ
20	80	5.6×10^3
40	60	6.9×10^3
60	40	8.3×10^3
80	20	8.5×10^3

In Fig. 2 the standard curve for creatinine, dissolved in ethanol-water (4+1), is shown. As seen, a straight line passing through origo is obtained within the concentrations investigated, thus in accordance with Lambert-Beer's law.

With *creatine*, dissolved in ethanol-water (4 + 1), no maximum was obtained and the absorbancy at 235 mμ was only 1.3 per cent of that of creatinine and thus neglible.

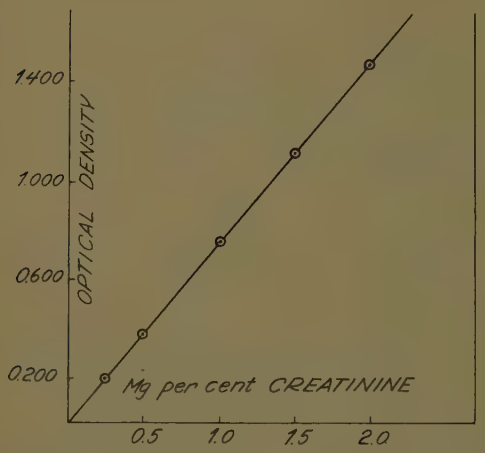


Fig. 2. Standard curve for creatinine, dissolved in ethanol-water (4 + 1). Beckman DU, light path 1 cm, 235 mμ. Solvent as blank.

SUMMARY

In methanol-water, ethanol-water and pure butanol, creatinine gives a distinct maximum in the ultraviolet region at 235—236 mμ.

With creatine in the same solvent no maximum was obtained.

A standard curve for creatinine in ethanol-water 4 + 1 following Lambert-Beer's law is presented.

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ROTEIN PATTERN IN POLYP FLUID AND CELLULAR INFILTRATION OF NASAL POLYPS

By P. BERDAL and B. OLHAGEN

om Rikshospitalet, University of Oslo, Dept. of Oto-Laryngology, Oslo, Norway, Konung V's Forskningsinstitut, Stockholm, Sweden, and Institutt for Generell og Eksperimentell Patologi, University of Oslo, Oslo, Norway

(Received for publication October 1, 1954)

patients with nasal polyps, Berdal found a markedly higher content of antibodies (reagins) in polyp fluid serum from the same patients. The the present investigation was to find ether or not this antibody accumula- as paralleled by a similar increase of globulin content.

study on plasma cells and globulin on, Andersen & Bing (1944) re- higher globulin content in polyp fluid serum in four out of twelve cases, significant difference in the eight re- patients.

MATERIAL AND METHODS

material consists of 25 cases of polyps asal cavity and accessory sinuses, from 150 cases registered during the April 1948 through March 1952. nimum amount of fluid necessary for horetic analyses was 2 ml, and the ng 125 patients had to be excluded, none of them provided samples nough to meet this minimum require- The majority of our 25 patients had mucous polyps, some pseudocystic alone (*5* a and b, *6, 11, 12*), and some ystic polyps concurrently with solid *0, 21, 22, 23, 24, 25*).

Polyp fluid was collected from solid polyps by squeezing, from pseudocystic polyps by puncture and aspiration. The fluid was generally thin, serum-like, coagulating 2—4 minutes after extraction. After removal of the fibrin clot, the fluid was clear, serum-like.

The electrophoretic analyses were performed in a Tiselius apparatus with inclined-slit optical device of Svensson. Sodium phosphate buffer, pH 7.6, ionic strength 0.1, with the addition of 0.15 mol NaCl was used. The serum and the polyp fluid were diluted to the same protein content (1.0—1.2 per cent according to the available amount of the specimen). The areas of the components in the pattern were measured planimetrically.

The method permits the measuring of a single component with an accuracy of $\pm$ 0.5 per cent of the total protein content.

The extirpated polyps were examined histologically, particularly with a view to the cellular infiltration. The specimens were fixed in formalin, and the following staining methods were used: hematoxylin-eosin, hematoxylin-erythrocin-safran (Masson), Pappenheim's. An approximate quantitative evaluation has been made of the total cellular infiltration, and the different types of cells. The total cellular infiltration was described as: ample, moderate and sparing respec-

Table I. *Comparison between protein analyses of serum and polyp fluid and cellular infiltration in polyps.*

Eosinophil granulocytes in nasal secretion and in blood, estimated age of polyps.

Case No.	Total protein content gm/100 ml	Protein in polyp fl. in per cent of protein in serum	Electrophoretic analyses: Albumin Abs. gm/100 ml	Albumin Rel. per cent	α globulins β globulins Abs. gm/100 ml	α globulins β globulins Rel. per cent	γ globulins Abs. gm/100 ml	γ globulins Rel. per cent	Difference in γ globulins Polyp fl.—serum Abs. gm/100 ml	Difference in γ globulins Polyp fl.—serum Rel. per cent	Cellular infiltration: Total	Relative: Lymph.	Plc.	Mcrph.	Neutr.	Eos.	Mastc.	Eosinophilia in nasal secretion	Eos. granulocytes in blood	Estimated age of polype in years
1 Polyp fl.	7.1	85.5	3.6	50.5	Abs. 0.9	Rel. 13.4*	2.6	36.1	+0.2	+ 7.6	Sparing	2/5	2/5	nil	few	few	few	—	108	4—5
Serum	8.3		4.0	48.5	0.5	6.6 1.4 16.4	2 4	28.5												
2 Polyp fl.	6.0	84.5	2.9	48.3	0.3	4.4 0.8 14.3	2.0	33.0	+0.6	+12.8	Ample	1/3	1/5	few	1/3	<1.5	few	—	87	2
Serum	7.1		3.8	53.8	0.7	9.7 1.2 16.3	1.4	20.2												
3 Polyp fl.	5.9	73.7	3.1	52.9	0.4	7.1 0.6 10.3	1.8	29.7	—0.4	+ 1.6	Moderate	1/2	1/5	nil	1/4	few	few	+—	862	2
Serum	8.0		4.0	49.3	0.8	9.8 1.0 12.8	2.2	28.1												
4 Polyp fl.	5.9	81.9	3.5	58.8	Abs. 1.0	Rel. 17.9*	1.4	23.3	—0.3	— 0.2	Moderate	1/5	1/5	nil	<1/5	2/5	few	+	153	2
Serum	7.2		3.8	53.0	0.4	6.1 1.3 17.4	1.7	23.5												
5a Polyp fl.	6.8	77.2	3.3	48.6	0.6	8.1 0.3 5.0	2.6	38.3	+0.7	+17.2								+—	228	2—6
Serum	8.8		4.7	53.4	0.7	7.4 1.5 17.3	1.9	21.9												
5b Polyp fl.	7.0	89.7	3.6	51.4	0.5	7.7 0.4 5.1	2.5	35.8	+0.7	+13.0										4/12
Serum	7.8		4.2	54.6	0.5	6.3 1.3 16.3	1.8	22.8												
6 Polyp fl.	6.9	84.1	3.7	54.2	0.6	7.9 0.6 9.0	2.0	28.9	+0.6	+12.2										1
Serum	8.2		4.9	60.2	0.6	7.2 1.3 15.6	1.4	16.7												
7 Polyp fl.	6.1	87.1	3.0	50.1	0.4	6.3 0.9 14.6	1.8	29.0	—0.1	+ 2.5	Ample	1/5	<1/5	nil	<1/5	3/5	few	+	239	8
Serum	7.0		3.5	50.4	0.5	6.6 1.1 16.5	1.9	26.5												
8 Polyp fl.	8.0	100.0	3.8	48.0	Abs. 2.9	Rel. 36.5*	1.3	15.5	?(Opalescence)		Ample	1/4	1/4	nil.	<1/5	2/5	sev	+	980	2—4
Serum	8.0		3.4	42.7	0.9	11.8 1.8 21.9	1.9	23.6												
9 Polyp fl.	7.7	84.6	3.2	41.9	0.6	7.1 0.9 11.8	3.0	39.2	—0.2	+ 4.0	Moderate	1/5	1/5	nil.	<1/5	2/5	few	+	194	6/12
Serum	9.1		3.9	42.9	0.8	9.2 1.2 12.7	3.2	35.2												
10 Polyp fl.	6.6	91.6	3.3	49.3	Abs. 0.8	Rel. 12.3*	2.5	38.4	+0.5	+ 9.8	Ample	1/5	2/5	nil.	few	1/3	sev	+	472	1
Serum	7.2		3.7	51.1	1.5	20.3	2.0	28.6												

	Serum	7.2		3.5	48.9		1.6		21.6	2.1	29.5											
14	Polyp fl.	6.4		3.2	50.9	0.5	7.3	0.9	14.5	1.8	27.3		Ample	2/5	1/5	nil.	few	1/3	few	+	766	2
	Serum																					
15	Polyp fl.	6.7		3.9	57.6	0.2	3.5	0.9	13.9	1.7	25.0		Moderate	3/5	1/5	nil.	few	<1/5	few	+—	344	6—7
	Serum																					
16	Polyp fl.	6.3	86.3	3.3	52.2	0.3	4.4	1.0	15.1	1.7	28.3	+0.1 + 6.3	Moderate	1/2	1/3	few	<1/5	few	few	—	272	1
	Serum	7.3		3.5	47.4	0.7	9.9	1.5	20.7	1.6	22.0											
17	Polyp fl.	6.6	94.2	3.6	55.0	Abs. 1.2		Rel.	17.9*	1.8	27.1	—0.3 —3.4	Ample	<1/5	<1/5	nil.	nil.	3/4	nil.	+	547	6/12
	Serum	7.0		3.3	47.1	0.4	5.0	1.2	17.4	2.1	30.5											
18	Polyp fl.	6.6	85.7	3.3	55.4	Abs. 1.3		Rel.	20.5*	1.6	24.1	—0.4 — 4.6	Sparing	1/3	<1/5	nil.	<1/5	few	sev	—	69	6/12
	Serum	7.7		3.4	44.3	0.4	5.2	1.7	21.8	2.2	28.7											
19	Polyp fl.	6.5	86.6	3.5	54.1	0.3	5.1	0.7	10.0	2.0	30.8	+0.5 +10.8	Ample	2/5	1/5	nil.	few	1/3	nil.	+—	67	3/12
	Serum	7.5		4.2	55.6	0.7	10.2	1.1	14.2	1.5	20.0											
20	Polyp fl.**	7.5	87.1	3.0	40.6*	0.3	3.5	0.6	7.8	3.6	48.1	+1.5 +23.2	Ample	2/5	2/5	few	<1/5	few	nil.	+—	100	2/12
	Serum	8.6		4.5	52.6	Abs. 2.0		Rel.	22.5*	2.1	24.9											
21	Polyp fl.**	5.5	73.3	3.1	56.1	0.3	6.0	0.7	12.7	1.4	25.2	—0.2 + 3.5	Moderate	1/2	1/4	few	1/5	few	few	+—	96	2
	Serum	7.5		4.2	56.3	0.7	8.8	1.0	13.2	1.6	21.7											
22	Polyp fl.**	6.4	100.0	3.2	50.0	0.2	3.7	0.9	14.7	2.1	31.8	+definite	Ample	3/4	<1.5	few	<1/5	few	few	+	160	1
	Polyp fl.	6.4		3.2	50.6	0.2	3.7	0.9	13.9	2.1	31.8	+definite										
23	Polyp fl.**	6.2	73.8	3.5	57.1	Abs. 0.9		Rel.	15.0*	1.8	27.9	—0.2 + 3.8	Moderate	2/5	<1/5	few	<1/5	2/5	few	+	502	1
	Serum	8.4		4.5	53.6	0.8	8.8	1.1	13.5	2.0	24.1											
24	Polyp fl.**	7.0	85.3	3.8	55.3	0.6	8.2	0.8	11.1	1.8	25.4	+0.2 + 6.5	Ample	2/3	<1/5	few	1/5	few	nil.		46	>1
	Serum	8.2		4.9	60.0	0.5	6.7	1.2	14.4	1.6	18.9											
25a	Polyp fl.**	6.7	91.8	3.9	58.9	0.5	7.0	0.3	4.7	2.0	29.4	+0.5 + 9.5									150	
	Serum	7.3		4.4	60.6	0.4	5.8	1.0	13.6	1.5	20.0											
25b	Choanal polyp	5.9	80.8	3.7	62.3	Abs. 0.9		Rel.	15.2*	1.3		—0.2 + 2.5	Sparing	1/3	1/3	nil.	<1/5	<1.5	few	—	150	1
	Serum	7.3		4.4	60.3	0.4	5.8	1.0	13.6	1.5												

*) Poor separation of α- and β-globulins
**) Pseudocystic polyps

Polyp fl. = Polyp fluid
Abs. = Absolute
Rel. = Relative
Lymph. = Lymphocytes
Plc. = Plasma cells
Mcrph. = Macrpohages
Neutr. = Neutrophils
Eos. = Eosinophils
Mastc. = Mast cells
Sev. = Several

tively. Examples are given in Figs. 1, 2 and 3. The amount of lymphocytes, plasma cells and granulocytes has been described as ample if the relative quantity was about one-fifth or more and below this limit as moderate or sparing.

With a view to the significance attributed to eosinophilia in nasal secretion and blood as an indicator for allergic genesis, the quantities of eosinophils in blood and nasal secretion were estimated. Smears from nasal secretion were stained by a modified Pappenheim method. In accordance with Andersen (1943) and others, eosinophilia of the secretion was diagnosed if the relative quantity was one-tenth or more of the leukocytes present, and when the eosinophils occurred in heaps even if the relative quantity was less than one-tenth. In blood the total number of eosinophils was counted by the method of Rud.

DISCUSSION

In case *8* the result of the analysis was considered less reliable due to opalescence of the polyp fluid, and it has been excluded from the discussion. It appears from the table that in 17 cases there is a higher concentration of gamma globulins in polyp fluid than in serum. In 12 cases the increase was both absolute and relative (*1, 2, 5* a and b, *6, 10, 11, 12, 16, 19, 20, 24, 25*), in 5 cases the increase was relative only (*3, 7, 9, 21, 23*). This relative increase, however, we have considered equally significant as the absolute difference between gamma globulins in polyp fluid and in serum, the absolute difference being masked by the generally lower total protein content in polyp fluid (except in case *11*). In cases *14, 15* and *22* the polyp fluid only has been examined, and of these case *22* undoubtedly represent absolute increase of the gamma globulin tent in the polyp fluid. This can be ded from its high value coinciding with patient's normal sedimentation rate, w excludes a massive gamma globulin inc in plasma. With regard to the other cases no definite conclusions could be dr Lower value of the gamma globulins i polyp fluid than in serum was registere three cases (*4, 17, 18*), and possibly in case *13*.

The high protein content in polyp and the relatively close correspondence the plasma protein pattern indicate polyp fluid is not a pure filtration transu Increased permeability of the smallest ve must be present. An identical opinion been advocated by Eichelberger & Lin (1941) with regard to fluid in non-secr cysts (pseudocysts). Analogous rela exist for joint exudate (Olhagen 1950). the other hand the protein content di qualitatively markedly from that of pla and the question arises whether produ of certain protein fractions, particu gamma globulins, takes place in the po The demonstration of antibodies in concentration in polyp fluid (Berdal 1954) strongly indicates a local anti production in polyps, and the high v of gamma globulins demonstrated here respond well with this assumption. A fu support of this view is the histologica monstration of lymphocytes and plasma in relatively large quantities, so that a s tural basis seems to exist for a conside local production of antibodies. It is re able to assume that similar relations for the allergically affected mucous

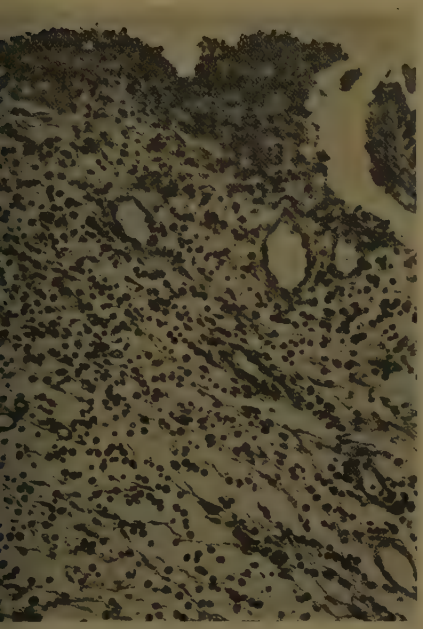

Fig. 1.

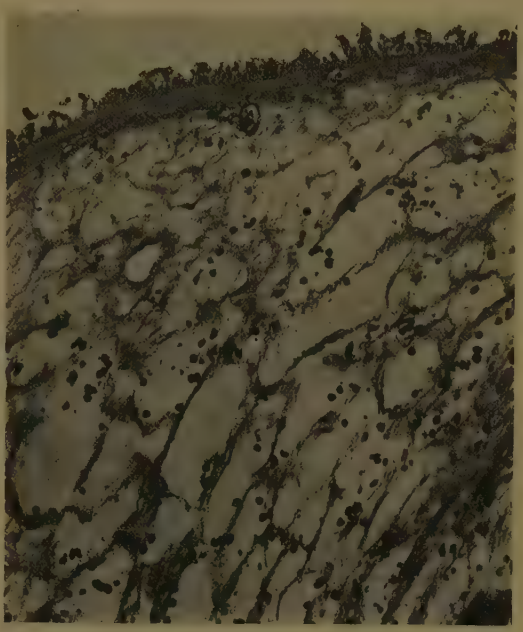

Fig. 2.

the nose and the accessory sinuses ole. In this connection it may be d that Thompson & Olsen (1950) nd experimental evidence for a local on of antibodies in the cornea.

igher content of gamma globulins fluid speaks against an influx of s produced somewhere else. A pas- ease of gamma globulins might be due to a selective and quicker re- n of the smaller protein molecules) from the tissue spaces of the A correlation then might be expected the gamma globulin content and the e polyps. As will appear from the wever, this is not so, for example ses 5 a and b, in which we had the ity of examining polyps of old as recent date from the same patient.

Additionally, in case of selective reabsorption one should expect relatively higher value of beta globulins than of gamma globulins because of the larger molecules of the former. On the contrary we have found the relative values of the beta globulins to be lower. This is particularly striking in cases *5* a and b, *12, 16, 19, 20.*

A surprising finding was a definite increase gamma globulins in serum in 11 cases (*1, 3, 7, 9, 10, 12, 13, 17, 18, 20, 23*). This might be interpreted as an increase of the circulating antibodies. This problem will be the subject of a more detailed investigation in a subsequent study. However, it is not possible to say whether the gamma globulin increase in serum is specific, *i. e.* due to antibody production, or merely a non-specific phenomenon analogous to the non-

specific gamma globulin increase in liver cirrhosis. It should be noted that in cases *9* and *12* the increase of gamma globulins in serum is probably due to the patients suffering from rheumatoid arthritis and keratoiritis respectively.

A comparison of serologically observed antibody titres (Berdal) with the values of electrophoretically separated gamma globulins, shows a marked quantitive discrepancy: in case *11* the content of antibodies was approximately 100 times higher in the polyp fluid than in serum, while the content of gamma globulins was only 3 times higher. Similarly in case *23* the content of antibodies was about 30 times higher in the polyp fluid than in serum, while the absolute gamma globulin content was higher in serum than in the polyp fluid. Also these observations strongly indicate an active local production of antibodies in the polyps.

These findings correspond with observations published by Levinthal (1938) showing that streptococcal agglutinins were demonstrable more frequently in joint exudate than in serum in patients with rheumatoid arthritis. Further, Svartz & Oldhagen (1946), Perlmann (1949), Olhagen (1950) found high gamma globulin values in joint exudate from patients with rheumatoid arthritis. However, the findings are not easily interpreted, as old cases of hydrops on a non-infectious basis may also show high gamma globulin values. It should be stressed that gamma globulins and antibodies are not synonymous conceptions, and that serological proof is required for using the term antibodies.

With regard to the remaining protein fractions (albumin, alpha and beta globulins), no absolute or relative increase is fou polyp fluid. In serum a beta globulin in of obscure nature has been found i cases (*8, 16*).

The content of fibrinogen in the fluid has not been examined for tec reasons. Certain macroscopic observ should be mentioned, however. In the rity of cases the squeezed polyp fluid a as the aspirated pseudocyst fluid has f a continuous massive coagulum in the of two-three minutes. The clot behav typical plasma fibrin coagulum. In two (*11, 25*) the clots were small and thin, ably indicating a low content of fibri

According to the opinion of most au the pseudocysts have developed in or mucous polyps by a merging of the filled tissue spaces (Finck 1927, McG 1928, Hansel 1929, Aschan 1950). histological picture in our cases con with this view. Serological observ (Berdal) and the results of our el phoretic analyses also indicate that th no principal difference between the two of polyps as *e. g.* in cases *22, 25.* N has any principal difference been d strated between the protein pattern fluid from choanal polyps and from or nasal polyps.

Of great interest, in a comparison be the results of the protein analyses a histological picture, is the occurren lymphocytes and plasma cells. The of research carried out in the course past decade indicate that lymphocytes as plasma cells are capable of producin bodies. As possible producers of anti should also be mentioned the histi which, however, are difficult to disti

roblasts without special staining
From a biochemical point of view
s mainly possess the characteristics
a globulins (Tiselius & Kabat 1939).
lier papers the lymphocytes were
d to be the most important anti-
ducing cells (Ehrich & Harris
ougherty, Chase & White 1944,
Grim, Mertens & Ehrich 1944, Kass
l others.
strong evidence has been given,
y Scandinavian investigators, that
na cells play a dominating role in
uction of antibodies (Bjørneboe &
1943, Bjørneboe, Gormsen &
st 1947, Fagraeus 1948 and others.
ion has recently again been directed
mphocytes through the paper pub-
1952 by Wesslén, who has succeeded
pure lymphocyte suspensions from
cic duct — in transmitting passively
n hypersensitivity as well as the
ctic type of allergy. He has also
servations which strongly suggest
lymphocyte, in the form in which it
ne blood stream, does not contain
bodies, but is capable of producing
es.
f 9 cases with absolute and relative
of gamma globulins in the polyp
e histological examination showed
asma cells (*1, 2, 10, 16, 19, 20, 25*).
wo remaining cases sparing plasma
e found (*22, 24*). In the five cases
howed only a relative increase of
globulins, a moderate or sparing
f plasma cells were present (*3, 7, 9,*
In three cases with low values of
globulins relatively few plasma cells
nd (*4, 17, 18*).

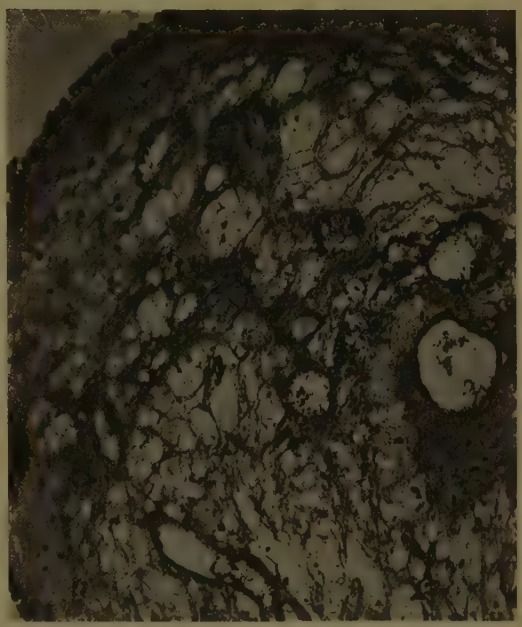

Fig. 3.

Lymphocytes occurred in relatively high numbers in all cases, except *17*. We have been unable to demonstrate any definite correlation between the content of gamma globulins and the quantity of lymphocytes. A small quantity of macrophages has been demonstrated in some cases.

The present investigations gave no definite answer to the question as to whether lymphocytes or plasma cells are the actual producers of antibodies. It is striking, however, that plasma cells, which under normal conditions are found only in small quantities in bone marrow and in blood, occur in relatively large quantities in the majority of cases with increase of gamma globulins in polyp fluid.

Small quantities or neutrophilic granulocytes were found in the majority of cases. Eosinophils occurred, on the other hand, in

ample quantities in half the number of cases examined, without any correlation to the gamma globulin content.

In the majority of cases mast cells were found, though in no great number. No correlation to the protein values was demonstrated.

We found no correlation between gamma globulins and the quantity of eosinophilic granulocytes in nasal secretion and in blood.

SUMMARY

The protein pattern of polyp fluid and serum from twentyfive cases of nasal polyposis has been analysed electrophoretically. Concomitant histological investigations of the cellular infiltration of the polyps were performed. High gamma globulin values in polyp fluid as compared with serum were demonstrated in two-thirds of the cases, partly with remarkably high values. As to the cellular infiltration an abundant occurrence of plasma cells was striking in the cases with high gamma globulin concentration.

The possible correlation between cell types and antibody production is discussed, and the probability of local production of gamma globulins in the polyps in stressed.

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PROTEIN STUDIES OF TRANSUDATES OF THE MIDDLE EAR

By L. A. CARLSON and T. LÖKK

King Gustaf V Research Institute, Stockholm, and the Department of Otolaryngology, Karolinska sjukhuset, Stockholm, Sweden

(Received for publication September 19, 1954)

nly serum and plasma but many rmal and pathological fluids of the ve been analysed by means of paper noresis, *e. g.* cerebro-spinal fluid ler & Wallenius, 1951), pleuritic (Keller 1954), urine (Kunkel & 1953), saliva (Kinersly 1953) and d perilymph of the inner ear (Jensen rup, 1954).

ransudates of the middle ear have alysed by means of paper electro- in a few cases by Ivstam (1952) wed that the components of serum were present in the transudates. , no quantitative relations were

MATERIAL

atients were generally school child- were sent from various places to rtment of oto-laryngology at Karo- jukhuset for the treatment of oto- is. Ear, nose and throat were thor- nspected in the usual manner and e tone audiometry was recorded.

g 1953—54 paracentesis was per- upon some 100 patients. The aspi- nsudates were analysed by electro- as soon as possible after the opera- r diverse technical reasons, mostly mixture in the transudate, only 17 nained for closer analysis.

METHODS

Collection of samples

The ear-drum was punctured with a specially constructed needle while the patient as a rule was under general anesthesia. The instrument (Fig. 1) consisted of a graded glass tube (B in Fig. 1, diameter 5 mm) connected with a needle (diameter 1.4 mm, A in Fig. 1) and rubber tubing (C in Fig. 1). The operator kept the rubber tubing between his lips when puncturing the ear-drum and as much transudate as possible was immediately aspirated into the tube. The tube was corked with a rubber stopper and centrifuged at 3500 rpm. Samples which after centrifugation showed any traces of red blood corpuscles were discarded, as only samples not contaminated with blood can be used as a basis of a judgment of the protein components in the transudates.

Determination of total protein concentration

The total protein concentration was determined according to Goa's modified biuret reaction (Goa 1953). Serum or transudate, 0.01 ml, was diluted with 0.9 per cent NaCl to a volume of 0.5 ml. Of this dilution 0.1 ml was taken for protein analysis and precipitated with trichloracetic acid. The precipitate was dissolved in 0.5 ml of the biuret reagent and the optical density read at 330 mμ after 15 minutes. The determinations were carried out in duplicate when there was a sufficient amount of the sample. Furthermore, the protein concentration was determined according to Kjeldahl in cases where a large quantity of transudate was obtained. The values obtained by this method showed a good correspondence to those obtained by the biuret reaction.

Paper electrophoresis

The paper electrophoresis was carried out according to Kunkel & Tiselius (1951) in veronal buffer of pH 8.6, and ionic strength 0.1. As a rule the potential was applied for about 15—20 hours. After that the paper was placed for 15 minutes in an ethanolic solution containing 1 per cent bromophenol blue and 10 per cent $HgCl_2$ (w/w) and then the paper was washed with 0.5 per cent acetic acid (v/v). This procedure gave a totally white background.

The quantitative determination of the electrophoretic components

The quantitative determination of the separate components was carried out by cutting out each spot and eluating the color with 5 per cent Na_2CO_3 in 50 per cent methanol. The eluated color was then determined spectrophotometrically at 595 mμ.

RESULTS

The transudates have been studied as to their appearance, their total protein concentration and their electrophoretic components.

The general qualities of the transudates.

The aspirated amounts of transudate varied from 0.02 to 0.2 ml, as a rule there was about 0.05 ml. The colors of the transudates varied from intense to very pale yellow. In some cases the transudates showed a faint greenish tint. No correlation between the color of the transudate and that of the corresponding serum could ever be seen. The viscosity varied from case to case but generally there was some stringiness.

The total protein concentration.

Throughout a high concentration of protein was found in the transudates. The transudates always contained more protein than the corresponding sera except i case where the concentrations were The average percentage of protein i transudates was 11.4 (w/v) varying 16.3 to 7.0 per cent), and in the se (w/v) (varying from 8.4 to 6.8 per (Table I).

In some samples of very viscous tra tes a slight precipitate appeared whe transudate was diluted with NaCl. N rection has been made for this preci

The protein components obtained by electrophoresis

As serum and transudate from the patient were taken simultaneously examined in the same way it was po to compare the mobility of the protein ponents of the transudate with that serum components. Generally the el phoretic mobility of the protein compo from the transudates corresponded t of the protein components of the (Fig. 2). Because of this fact it seems to assume that the protein components transudates are practically identical those of the serum.

In very viscous transudates the re mobility of all the components was s down about 10 per cent. In all prob this was due to the high viscosity.

In about one third of the analysed there appeared a component moving sl faster than the albumin.

The intense yellow color found in so the transudates could be followed the electrophoresis and was found associated with the albumin. We a this yellow substance to be bilirubin.

The results of the quantitative dete tion of the different components are

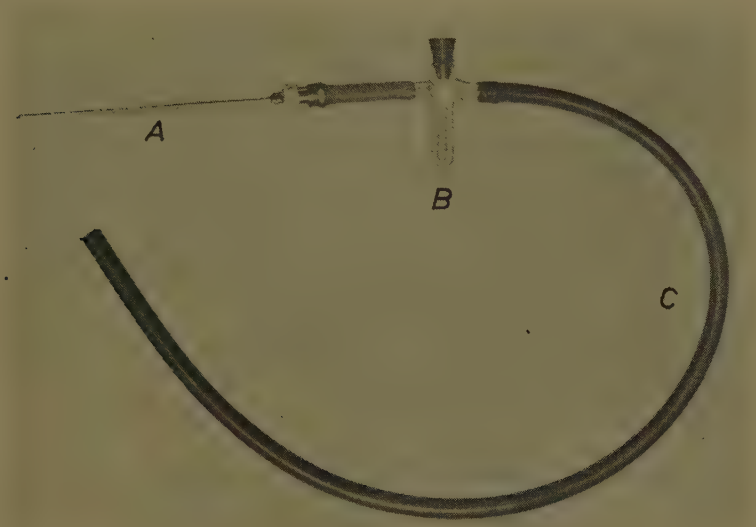

Fig. 1. The instrument for the aspiration of the transudate. A is the needle, B a graded glass tube and C rubber tubing.

I. *Mean values of the total protein ıtion of the sera and the transudates in per cent (w/v).*

Serum	Transudate
7.6 ±0.51*	11.4 ± 3.29

ard error calculated according to the $=\pm\sqrt{\frac{\Sigma a^2}{n-1}}$ where a = the deviation mean value and n = number of cases.

II. The cases have been divided groups, the first consisting of cases g duration of the disease and the ne with short duration. The first cludes 13 cases and the second 4

comparing the percentage distribu- e different protein components, we convenient to use the term *ratio.* By this we mean the ratio between the percentage concentration of a protein component in the transudate and the percentage concentration of the same protein in the serum. It is evident from Table II that the cases of short duration showed high ratios for the albumin fraction and low ratios for the α_2- and γ- globulins.

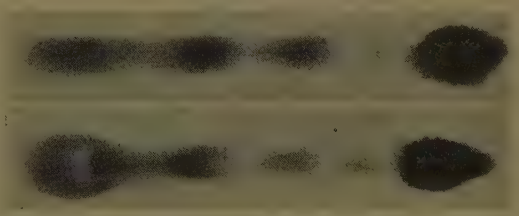

Fig. 2. Stained paper electrophoretic strip showing serum (above) and transudate from a case of long duration. The components are (from left to right): γ-, β-, α_2- and α_1-globulin and albumin. The extended area of the γ-globulins in the transudate is clearly seen.

Table II. *The mean values of the percentual distribution of the electrophoretic pr components in sera and transudates.*

	Cases of long duration			Cases of short duration		
	Serum	Transudate	Statistical † Significance	Serum	Transudate	Statistic Signific
Albumin	69.5 ± 3.63*	62.5 ± 6.27	<0.1%	58.5 ± 4.04	70.7 ± 1.32	<0.2%
α^1-globulin	3.3 ± 0.16	3.3 ± 1.01	insignificant	3.9 ± 0.38	3.1 ± 0.32	insignific
α^2-globulin	7.6 ± 0.31	6.4 ± 0.92	insignificant	10.2 ± 2.34	3.7 ± 0.54	<0.2%
β-globulin	8.6 ± 0.37	8.5 ± 0.33	insignificant	9.9 ± 0.54	8.1 ± 0.98	insignific
γ-globulin	11.2 ± 0.58	19.1 ± 1.32	<0.1%	17.5 ± 1.72	12.4 ± 1.76	<1%

* Standard error, calculated according to the formula $\sigma = \pm\sqrt{\frac{\Sigma a^2}{n-1}}$ where a = the devia from the mean value and n = number of cases. † Calculated according to the t-test.

On the other hand cases of long duration had a low ratio for the albumins and a high ratio for the γ-globulins. There was no difference regarding the other globulins.

DISCUSSION

The probable explanation of the high concentration of protein in the transudates is that after the primary transudation, water resorbtion takes places. This has earlier been suggested by Ivstam (1952).

However, it is evident that not only is water resorbed but also that other mechanisms seem to be active during the formation of the transudate. This can be concluded from the fact that there is no direct relationship between the protein pattern of serum and that of transudate.

The following mechanisms may con to this fact:

1) Different transudation and reso rates of different protein components.

2) A synthesis or a destruction *in* one or several protein components.

As to the first possibility, the co tively small albumin molecules shou out more easily than the other protei ponents through the capillaries duri transudation. In this way the ratio o min should be relatively larger in the phase of the disease. In Table II it is that this is actually the fact in the with short duration of the disease.

Furthermore it is likely that thes tively small protein molecules will b easily resorbed during the resorption

so apparent that the cases with long
n had a low ratio for the albumin
.

ew of the size of the molecules it is
difficult to draw conclusions with
to the globulins, as the serum globu-
, in fact, of heterogenous composition
ithin each elctrophoretically defined
ent.

α_2-globulins seem to have a high
nolecular weight of about 300 000.
ight explain the low ratio for these
is in the acute phase of the transuda-
able II).

ratio of the γ-globulins was low in
ansudates and high in long standing
ates. In some old transudates the γ-
concentration had increased enorm-
ne maximum ratio being 3.2. It seems
explain these *excessive* increases by
absorption of the γ-globulins, at least
onsidering the size of the molecules.
e of the analysed old transudates the
ins appeared to be qualitatively dif-
rom those in the corresponding sera.
ot representing the γ-globulin was
tended, *i.e.* showing a greater hetero-
than normally (Fig. 2). This may
the presence in some transudates of
ulin different from that circulating
lood.

circumstance and the great amounts
bulins found in some old transudates
d above may imply a local synthesis
bulins in the middle ear. In connec-
h this it is worth mentioning that
synthesis of γ-globulins has been
the aqueous humor (Thompson &
950) and in nasal polyps (Berdal &
, 1955). Furthermore it is known
that regenerative and chronically fibrous processes lead to a stimulation of γ-globulin synthesis.

As to the α_1-globulins and the β-globulins, there does not seem to be any relationship between their presence in the transudates and the duration of the salpingitis.

The pre-albumin component does not seem to be related to the duration of the disease or to the total amount of protein in the transudate. In cerebro-spinal fluid, on the other hand, such a relationship seems to exist according to Griese, Aly & Oldershausen (1953).

Fibrinogen has not been observed in any of the analysed transudates. In connection with this it is worth mentioning that quite fresh joint exudates contain fibrinogen while long-standing exudates do not contain this component (Olhagen 1950).

It would, of course, be of greatest interest to follow the protein composition of the transudates with regard to their age. However, this problem can probably be solved only by studies of experimentally developed transudates with a known time factor.

Investigations in this direction are planned.

These investigations will be continued by one of us (T. Lökk) for further elucidation of other problems on this subject.

SUMMARY

1) The total protein concentration in the transudates was higher than (or equal to) the serum protein concentration. The most probable explanation of this fact is that water is resorbed faster than protein during the resorption phase.

2) All the serum protein components have been found in the transudates. On the other hand fibrinogen could not be found in the analysed samples.
3) There was no simple relationship between the electrophoretic protein pattern of the transudate and that of the corresponding serum.
4) In cases with short duration of the disease the percentage concentration of the relatively small-molecular albumin was found to be higher in the transudate than in the serum and in long-standing cases the reverse was found to be true. The explanation of this has been discussed.
5) In the transudates a low percentage concentration of the γ-globulins (as compared with serum) was found in the early phase of the disease, but in long-standing cases this concentration increased greatly. These findings have been discussed and the conclusion was drawn that the increasing concentration of the γ-globulins in old transudates is probably due not only to filtration and resorption mechanisms.
6) In one third of the cases a protein component has been found that can not be found in normal serum. This component moves somewhat faster than the albumin.

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SPECTROPHOTOMETRY ON WHOLE BLOOD

By W. FALHOLT

From the Medical Clinic B, Rigshospitalet, University of Copenhagen, Denmark

(Received for publication October 14, 1954)

lich, Heimbecker & Bing (1950) and nadle, Ferguson, Gregg & Gilford have constructed apparatuses for us registration of Evans blue dye e blood. These apparatuses are sen- changes in oxygen saturation of the mpled, which is of no great import- en large amounts of Evans blue dye cted into the blood stream, so that entrations of dye recorded are high. sing the dye method for determina- cardiac output or residual volume of n the right heart ventricle (Bing, cker & Falholt, 1951), it is of value le to make several successive deter- ns, and this has hitherto been limited, undesirable to inject more than 60— f Evans blue dye into humans within of weeks.

ossibility of repeated successive de- ions of cardiac output or residual by means of a continuous recording after injection of small amounts of lue dye, is thus based upon the suc- utomatic correction for changes in saturation of the blood sampled (Fal- 53). In the search for a method of ing this correction a study of the light absorption of whole blood was performed.

Kramer (1934, 1935), Matthes (1934), Matthes & Gross (1939) and Drabkin & Austin (1932) have proved that Lambert-Beer's law is applicable to solutions of hemoglobin of high concentration and to whole blood. A new factor, however, is introduced when spectrophotometric measurements are performed on whole blood, as this is a heterogenous system, containing blood corpuscles.

According to Kramer, the loss of light in whole blood is due to absorption in the hemoglobin of the erythrocytes and in the structure of the erythrocytes, but furthermore a loss of light will occur due to reflection and scattering of light. Kramer determined the total absorption (composed of 1. absorption by the pigment alone, 2. reflection, and 3. scatter of light) photoelectrically, using the Winkel-Zeiss monochromator in the red and near infrared region (600—680 mμ). He found that the reflection, like the absorption, is a logarithmic function of the oxygen saturation of the blood.

The factor of scattering of light in turbid media was first explored by Raileigh who, as early as 1871, studied the scattering of

light by fog and found that the intensity of the scattered light varied inversely as the fourth power of the wave length, when the particles are small compared to the wave length.

Houghton (1931) and Stratton & Houghton (1931) studied the transmission of light through artificial fog and concluded that the transmission of light is dependant on the particular particle size or sizes of the fog on which the measurements are made. They furthermore proved that Raileigh's formula was not applicable to scattering of light by particles as large as certain artificial fog drops. Further studies on the scattering of light by particles, small compared to the wave length, have been carried out by Barnes & La Mer (1946) andDoty & Edsall(1951).

Drabkin & Austin (1932) and Drabkin (1950) have made spectrophotometric measurements on solutions of hemoglobin, hemolysed blood and native blood in the visible region of the spectrum. Drabkin maintained that due to variations in the number and possibly size of the red cells, reproducibility of absorption data from blood to blood can hardly be expected, and he concluded that the absorption spectra of native non-hemolysed blood are the effects of extreme turbidity. Compared to the absorption curve of hemolysed blood, Drabkin found that the absorption curve of non-hemolysed blood is flattened due to the relative greater absorption at the red and blue ends than in the mid-region of the visible spectrum.

In 1939 Drabkin & Singer studied the scattering of light by suspensions of particles and found that in turbid suspensions the total incident light, I, may be considered broken into 3 components: 1) a fraction is transmitted ,T_1, 2) one is scattered, T and one is reflected, R, and he gives th mula:

$$T' + R + A = I$$

where $T' = T_1 + T_2$, and A is the absorbed.

Drabkin finds that 1) the scatteri light is a function of the size and dens the particles, 2) scattering is also a fu of the wave length, a greater effect observed at shorter wave lengths. How in his results of measuring the scatteri light in the visible region of the spe by whole blood, Drabkin found an inde ence in the range of wave length from 500 mμ.

Drabkin determined the spectrop metric extinction due to scattering, e_s, particles with and without hemoglobin a and suspensions of erythrocytes in re to the wave lengths used and numb particles per ml. In suspensions of ery cytes it was found that the total extin coefficient, e_t, could be expressed b equation:

$$e_t = e_s + e_p \cdot f_1 (N \cdot d)$$

where d is the depth of the cuvette, number of particles. e_p, is the extin coefficient of the pigment alone. The tion f_1 (N. d) was empirically deter e_s is also a function of the number o ticles. By this formula Drabkin was a determine the spectrophotometric extin in whole blood due to scattering, e_s, in tion to the wave length of light use number of particles per ml, N.

According to Kramer, Elam, Saxt Elam (1949), Lambert-Beer's law, no

xtures of hemoglobin and oxyhemo- can be expressed as follows:

$$= \frac{1}{(e_2 - e_1) \cdot d} \cdot \ln I/I_0 + \frac{e_2 \cdot c}{e_2 - e_1}$$

c_1 is the oxygen content of hemo- and c the oxygen capacity of hemo- e_1 is the extinction coefficient of noglobin, e_2 the extinction coefficient oglobin. I/I_0 the transmission of light. his equation plottings of c_1 against e linear with a slope of $\frac{1}{(e_2 - e_1) \cdot d}$ capacity and any depth of cuvette, slope was not constant, as e_1 was to increase with increased capacity. ason for this is unexplained. Sekelj cently confirmed these findings (per- ommunication).

ecker (1953), on examinations of hemolysed human blood, has proved her blood constituents than hemo- make a neglegible contribution to the osorption in the visible region of the n. In the infrared region hemolysed as found to have a somewhat higher ion than a solution of pure hemo- of the same concentration.

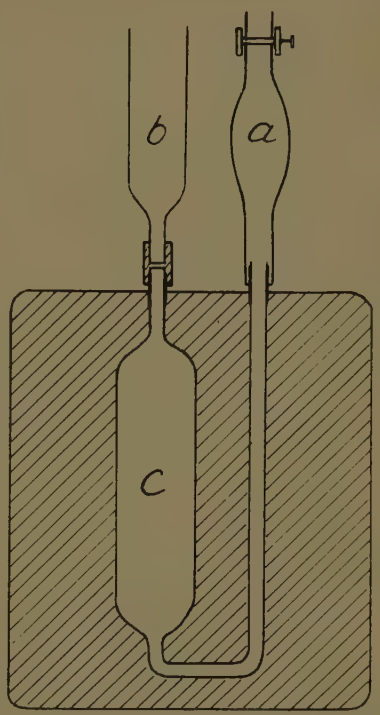

Fig. 1. Sketch of the cuvette as seen in the direction of the light. — The cuvette is constructed in plexiglass and consists of 2 symmetrical halve. In the plane surface of one half is engraved the cuvette opening, c, and the canals leading the blood to and from the cuvette. The cuvette opening is 0.1 mm deep. The 2 halves of the cuvette are kept together by clamps at the edge of the cuvette.

TECHNIQUE

was withdrawn into an oiled syringe, con- few crystals of calcium oxalate, from an after prolonged stasis so that the oxygen n of the blood sampled was low. Succes- ood was withdrawn and saturated by rota- pure oxygen. After shaking the samples gen saturation and capacity were deter- the van Slyke apparatus. The blood was sferred into a specially constructed cuvette t of plexiglass, which could be adapted to man Mod. D. U. spectrophotometer. The depth of the cuvette was 0.1 mm. The cuvette was filled with the unsaturated portion of blood through the rubber tube, marked a in Fig. 1. When the blood was halfway up in the chamber, b, the rubber tube, a, was locked by a clamp. The surface of the blood in the chamber was covered with a layer of paraffin oil, and the upper part of the blood, which had been in contact with air, was removed through a cannula inserted into the chambre.

Absorption data were then determined in fully saturated whole blood, in unsaturated blood, and finally in hemolysed blood. Readings of transmission of whole, non-hemolysed blood were first

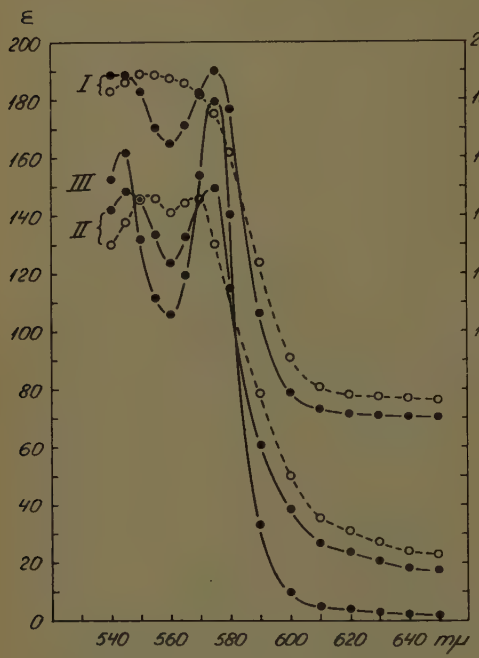

Fig. 2. Extinction curves of whole blood. Curve 1 illustrates the extinction, when parallel light was used, curve 2, when diffused light was used for the determination. The figure of extinction given is the density of blood, containing 16.2 grams of hemoglobin per 100 ml, in a layer of 1 cm.

—●—●—●— fully saturated blood.

—o—o—o— unsaturated blood, curve 1, 33 per cent oxygen saturation, curve 2, 52 per cent oxygen saturation, curve 3, hemolysed blood.

The figures of extinction in the green part of the spectrum in curve 1 are in agreement with those given by Matthes.

obtained with parallel light falling through the cuvette. After this the light, before entering the cuvette, was diffused by means of a white celluloid filter, attached to the light side of the cuvette. As the light passing the cuvette in this way is practically totally scattered in the direction of light, no further scattering of light can occur during its passage through the blood. Likewise diffused light is used for the 100 per cent standard transmission reading. The slide width and sensitivity of apparatus were adjusted accordingly.

Before each reading was performed, the blo the cuvette was brought into streaming by pressing the rubber tube, a, Fig. 1. Errors d sedimentation of the erythrocytes were thus av

When parallel light was used, the transmi of whole blood was compared to that of a filter transmitting 8 per cent of light. The se vity and slide width of the Beckman appa were adapted so that the black filter gave a t mission figure of 40 per cent. This sensitivi the apparatus resulted in a fictive transmissi the fully saturated blood of 99 per cent a wave length of 650 mμ. In this way the scale of the Beckman apparatus could be used the measurements made more exact. The cor data of density obtained in this way are giv Fig. 2.

DISCUSSION

The strong absorption of whole b makes it necessary to examine very layers of blood in order to obtain measurements of transmission. Fur more, an apparatus with suitable sensi and quality of monochromatic light is n sary to get exact results.

The extreme density of whole bloo as mentioned above, due to 3 factors absorption by the hemoglobin and stru of the blood corpuscles, reflection and tering of light. From these facts it m understood that when parallel light thr the cuvette is used, the figure of transmi read will be very different from one ratus no another, dependent upon the of the cuvette, the slide width and, abo the distance from the cuvette to the phot Because of the scattering of light b heterogenous system, the intensity o scattered light behind the cuvette decrease inversely with the square o distance from the cuvette. As whole

r of 0.1 mm does not scatter the light the directly transmitted light will be asing percentage of the total light d, with increasing distance from the As, furthermore, the scattering of whole blood is dependent upon the size and shape of the red blood es, it may be seen that the figures ction of whole blood, when passed by light, will hardly be reproducible, in nce with Drabkin.

rror of extinction coefficients deter- vith parallel light is illustrated by the ced difference between these and easured, when diffused light was used measurements. The extinction coef- given in Fig. 2, curves 1 and 2, are rage of repeated measurements per- on the same blood sample. The varia- the figures of single readings were per cent between the vawe lengths mμ to 580 mμ, within 15 per cent 0 to 540 mμ. As expected, it was vious that even the slightest sedimen- f the red blood corpuscles in the gave wide deviations of transmission s.

Drabkin, it was found that there was ite change in the scattering of light e blood in the range of wave length 0 mμ to 540 mμ as the small pro- l differences in scattering effect be- urves 1 and 2, Fig. 2, are within the f the measurements.

comparison of the hemolysed blood ig. 2, curve 3, with the whole blood hows that the latter are smoothed out, probably the result of the heterogeniety of whole blood.

Sekelj (personal communication) has measured the extinction of whole blood in continuous streaming, and found that the figures of extinction varied with the rate of flow through the cuvette, which is explained as the result of changes in scattering effect of the blood. This phenomenon has not been found here, probably the result of perfect mixing in the cuvette when the measurements were performed.

SUMMARY

In constructing an apparatus for continuous registration of concentrations of Evans blue dye in whole blood, a study on the absorption of light by whole blood was performed.

A method is described, by means of which it was possible to determine continuously the extinction coefficients of whole blood, after correction for scattering of light, in the range of wave length from 650 mμ to 540 mμ. The extinction coefficients of whole blood obtained in this way are the expression of the absorption of the constituents of the blood and the reflection. The importance of correction for scattering effect, when extinction coefficients of whole blood are determined, is illustrated by the comparison of extinction curves, determined with parallel light and diffused light through the blood.

The application of the present findings to the construction of a whole blood colorimeter for continuous registration of Evans blue dye will appear in a later communication.

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FIBRINOLYTIC ACTIVITY IN HUMAN TEARS[1]

By O. STORM

From the Biological Institute, Carlsberg Foundation, Copenhagen, Denmark

(Received for publication Nov. 20, 1954)

resence of a plasminogen activator
n urine (Astrup & Sterndorff, 1952)
an milk (Astrup & Sterndorff, 1953)
ention to a possible fibrinolytic acti-
ne secretion from glands with narrow
y ducts. This paper shows the pre-
human tears of a fibrinolytic system
ng that recently found in human
Müllertz & Lassen, 1952) and in
nilk (Astrup & Sterndorff, 1953).

ETHODS AND MATERIALS

lytic activity was measured by the
fibrin plate method (Astrup & Müllertz,
which the substrate (bovine fibrin) con-
ninogen, and was recorded as the product
of 2 diameters of the lysed zone (average
erminations). The fibrinolytic enzyme
was estimated by means of the heated
e method (Lassen, 1952), in which the
en in the substrate was destroyed by
ne fibrin plates at 85° for 45 minutes.
these two methods it was possible to
between plasminogen activator and
enzymes proper. Activators produce
e standard fibrin plates by activation of
inogen of the substrate, but are inactive
eated fibrin.

plasminogen was prepared from ammo-
te precipitated fibrinogen (using 0.9 per
instead of the usual barbital buffer) by

inary communication: 4th European
gical Congress, Amsterdam Sept. 1953,

heating for 10 minutes at 54° C followed by dialysis of the supernatant against saturated ammonium sulfate. The precipitate was redissolved, dialysed against borate buffer, and lyophilized.

Streptokinase (Varidase) was obtained from the Lederle Laboratories.

The pH of the tear fluid was measured by glass electrodes (micro). The following buffers were used: sodium diethylbarbiturate buffer pH 7.75; glycocol buffer (Sørensen) pH 1.2—3.7; phosphate buffer (Sørensen) pH 9.5—11.1; all at ionic strength 0.05.

In the thermostability experiments the tear fluid was adjusted to the appropriate pH-values by n/1 HCl and n/1 NaOH and after heating neutralized with sodium bicarbonate or potassium phosphate (primary) in substance.

Tear fluid

Tears from patients with conjunctivitis and tears produced by irritation with chemical compounds were considered unsuitable because they were contaminated by leukocytes and epithelial cells. Emotional tears were therefore used. Generally they were centrifuged and the clear supernatants were used fresh or they were pooled and stored a few days in the icebox until a sufficient amount was collected.

RESULTS

Fresh untreated human tears produced fibrinolysis in a normal fibrin substrate. The tear fluid was diluted with sodium-diethyl-barbiturate buffer (pH 7.75) and the fibrino-

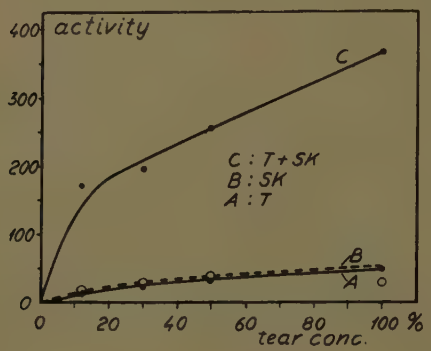

Fig. 1. Action of streptokinase on human tears. *Abscissa:* final concentrations in per cent of stock solutions. *Ordinate:* fibrinolytic activity on standard fibrin plates. Curve B — serial dilution of streptokinase (stock solution 0.002 mg/ml). Curve A — serial dilution of tears with barbital buffer. Curve C — serial dilution of human tears containing equal amount of streptokinase (in conc. 50 per cent of stock dilution).

lytic activity was determined on standard fibrin plate. The activity (diameter product of lysed zone) was plotted against the concentration of the tear fluid (in per cent of stock solution) (Fig. 1, curve A).

Streptokinase transforms a proactivator in blood and milk to an activator, which converts plasminogen to plasmin, (Müllertz & Lassen 1952, Astrup & Sterndorff 1953). The presence of a similar proactivator in human tears was demonstrated in the following way. By addition of streptokinase — a very weak solution of streptokinase (0.002 mg/ml) was chosen — to an equal amount of tears, a pronounced increase in the fibrinolytic activity was obtained (Fig. 1, curve C) in normal fibrin substrate. Streptokinase in corresponding concentration was only slightly active (Fig. 1, curve B). On heated fibrin plates a mixture of streptokinase and tears showed no activity.

If tears were placed on heated f plates, there was no fibrinolysis at all. if tears and bovine plasminogen (0.2 mg were placed on heated fibrin plates a d tion of the substrate was obtained (F curve A). Addition of bovine plasmin solution to the mixture of streptokinase tears (mentioned above) produced a ren able increase in the digestion (Fig. 2, c B. The controls 1) streptokinase, 2) b plasminogen, 3) bovine plasminogen+s tokinase, showed no fibrinolytic activit this experiment.

It appears from these results that do not split a plasminogen free subs

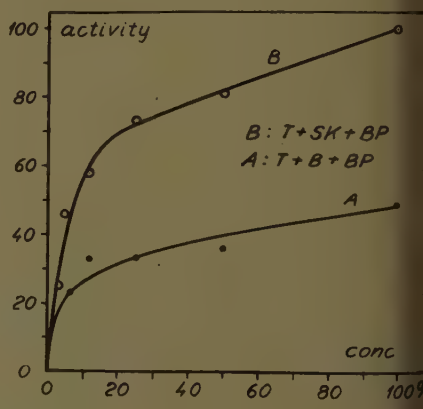

Fig. 2. Effect of plasminogen on the fibri activity of streptokinase activated human *Abscissa:* final concentration of plasmino per cent of stock solution (0.2 mg/ml in b buffer). *Ordinate:* fibrinolytic activity on fibrin plates. Curve A: serial dilution o minogen with diluted (barbital buffer) human (50 per cent). Curve B: serial dilutions o minogen (½ ml) with ½ mil. mixture of tears + equal amount streptokinase (stock tion 0.002 mg/ml). Controls: human tears, tears + streptokinase, streptokinase or plasm alone: no activity. Plasminogen + streptok trace of activity (25 mm²).

fibrin), but show a low fibrinolytic on plasminogen containing substrate late or heated fibrin plate + bovine gen). This activity on ordinary n be considerably increased by addi- streptokinase to the tears. Evidently fluid contains a small amount of a gen activator and a large amount ecursor, which can be transformed tokinase to a plasminogen activator. urther identification of the activator oactivator its heat stability was

eries of experiments it was attempted y the stability of the spontaneous r at different pH values. However, ility changed from experiment to ent, and with different samples at temperatures. Especially at low pH the results were unreproducible: es a high activity was found and es no activity at all remained after This was probably due to variation nts of an inhibitory substance, which l the enzymatic digestion by trypsin d fibrin. The inhibitor appeared to royed at very low pH (1—2) by (50° for ½ hour).

variable stability might also be due ent degrees of activation of the pro- in tears to activator at the different tures and pH values. When all the ator was transformed into activator s of streptokinase, it was possible to curves which could be reproduced periment to experiment.

fluid was incubated with strepto- 0.1 mg/ml) for ½ hour and diluted with sodium diethylbarbiturate buf- his diluted solution the concentration

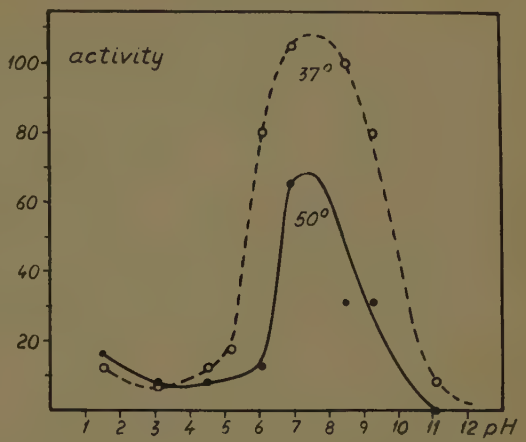

Fig. 3. Temperature stability of streptokinase activated human tears. *Abscissa:* pH values. *Ordinate:* fibrinolytic activity on standard fibrin plates in per cent of the stock solution activity at 20°. pH 7.8. Curve A: aliquots of stock solution adjusted to different pH, heated for ½ hour at 37°, cooled and neutralized. Curve B: aliquots of stock solution adjusted to different pH, heated for ½ hour at 50°, cooled and neutralized. Stock solution: 1 ml human tears — 1 ml streptokinase (0.1 mg/ml) incubated ½ hour, diluted with barbital buffer to 5 ml.

of the trypsin inhibitor was so low that it could not be measured. Aliquots were heated at 37°, 50°, 70° and 100° C at different pH values (pH 1.5—11), cooled to 0° (icebath) and neutralized to original pH of the tears (pH 7.8—8.2). The remaining activity was determined by the fibrin plate method. At 100° C all the activity was gone, and at 70° ther was practically nothing left. Curves obtained at 37° and 50° are shown in Fig. 3.

The curves obtained indicate that the tear activivator is a more labile compound than is the activator found in urine (Astrup & Sterndorff, 1952). It appears more similar to the compounds produced by activation with streptokinase in blood (Müllertz, 1953).

DISCUSSION

The presence of a plasminogen activator and its precursor in human tears may have a physiological significance. Like the activator in urine and milk, this fibrinolytic system in tears make a rapid resolution of clotted fibrin in the fine tearcanals (excretory ducts and lacrimal ducts) possible.

The fibrinolytic systems in blood, milk, urine and tears appear very similar if not identical.

The presence in tears of a bacteriolytic enzyme, lysozyme, has been known for years, but lysozyme did not digest ordinary fibrin plates and was not activated by streptokinase.

SUMMARY

1. Emotional human tears contain amounts of a plasminogen activator large amounts of a proactivator, is converted into a plasminogen acti by streptokinase.
2. The activator system in tears is similar to the fibrinolytic enzym human blood and milk.

ACKNOWLEDGEMENT

This study was aided by a grant to Dr. Astrup from the Josiah Macy Jr. Found New York.

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...GE OF DEXTRAN MOLECULES OF VARYING SIZES FROM BLOOD TO LYMPH

A PRELIMINARY REPORT

By G. GROTTE

From the Department of Clinical Chemistry and the Institute of Physiology of Upsala, Sweden

(Received for publication Dec. 1, 1954)

...0 when studying the passage of ...rom blood to lymph and urine, we ...Knutson & Bollman 1951) found ...ıtrations in both the last two fluids ...rsely proportional to the molecular ...tran. We were then using fractions ...n, the average molecular weights ...were determined viscosimetrically, ...of molecular sizes in each fraction, ...being unknown. With a turbidi... ...ethod developed for dextran by ... (1954), where molecular ranges ...ı in minute concentrations of serum ... lymph samples could be analyzed, ...n possible to extend these studies.

TECHNIQUE

...ıtalized mongrel dogs, various dextran ... of molecular weight ranges from ...00 were injected intravenously. Lymph ...re obtained through polythene tubing. ...g fractions of low molecular weight ... kidneys were clamped to prevent rapid ...by this route. Concentration of dextran ...nd lymph was determined with the ...agent (Grotte 1951, Wallenius 1954). ...weights were determined turbidimetri... ...enius 1954). All experiments here re... ... carried out on the "peripheral" lymph ...g of the dog (cf. Drinker 1941).

RESULTS

In earlier studies (Grotte *et al.* 1951) it was found that dextran fractions of large average molecular weights could penetrate to lymph; this was thought presumably to be due to "the low molecular tail" of each preparation. It has now been shown that dextran of molecular weight less than about 6,000 rather freely penetrates the "the blood — lymph barrier", attaining a concentration ratio C_L/C_B of about 1 in the steady state. For dextran of 10,000—40,000 mol. weight an increasing restriction of the barrier to increasing molecular dimensions appeared, the concentration ratio C_L/C_B tending to lower and lower values (Fig. 1). On the other hand, fractions of larger molecular weights from 60—350,000 show low lymph/plasma ratios, which do not vary with the mol. weights (Fig. 2).

From studies on proteins in blood and lymph it has been assumed that the passage of the larger proteins through capillary walls is more restricted than that of albumin (Drinker 1941, Perlmann, Glenn & Kaufman 1943, Cope, Graham, Moore & Ball 1948, Wunderly & Wuhrmann 1953). However, by using a slightly modified technique of paper electrophoresis we found albumin/globulin ratios essentially the same in blood

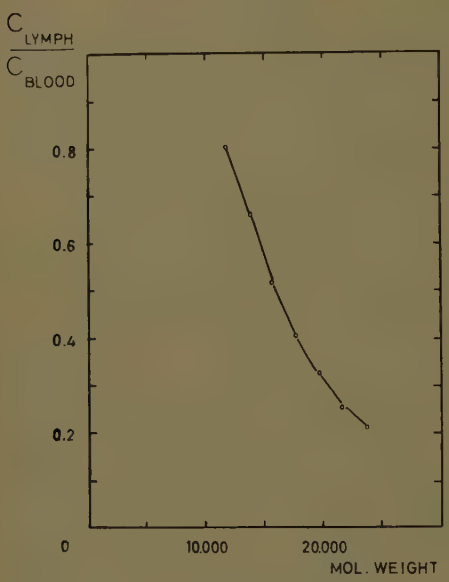

Fig. 1.

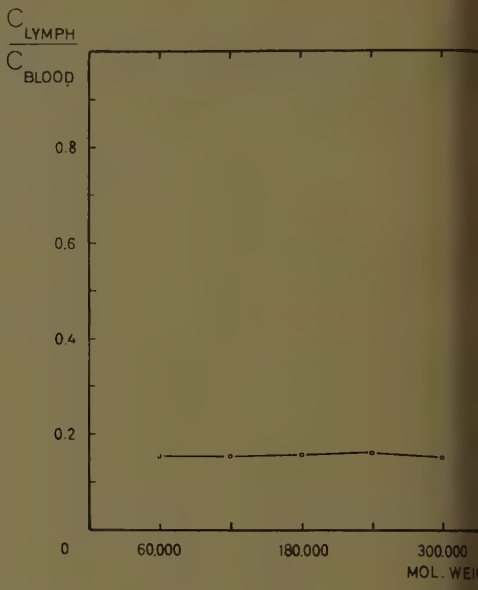

Fig. 2.

Steady state concentration ratios of different dextran molecules across the blood-lymph ba

and lymph, within the experimental errors (unpublished experiments).

If the pore theory of capillary permeability is applicable to "the blood-lymph barrier", the obtained results could be explained by assuming two different pore systems; capillary pores and capillary "leaks":

1. *Capillary pores* of a fairly large number and as suggested by Pappenheimer (1953) with a radius of 30—45 Å, though according to the present data completely restricting the albumin molecule (R_{ES} = Einstein — Stokes Diffusion Radius = 35 Å) and a dextran molecule of more than 20,000 mol. weight (R_{ES} = 35 Å). The process of exchange in these capillary pores may be dominated by diffusion.

2. *Capillary "leaks"*, few in number, but of a magnitude allowing the escape of occasional blood cells, and at normal filt rates giving no selective restriction t large molecules as globulins and dextr 350,000 mol. weight (R_{ES} = 125 Å process of transport being here one o in bulk.

As an explanation of the presence bulin and fibrinogen in lymph, Cohn pointed out the similarity of the equ diameters of the albumin, globuli fibrinogen molecules, differences in being mainly one of molecular A similar shape of the dextran molec been suggested (Ingelman & Halling However, recent work (Ogston & 1953, Edsall 1953) gives reason to dextran as well as protein molecules tion as more or less spherical particle necessitates the assumption of "leaks".

ACKNOWLEDGMENT

preparations were kindly placed at my
AB. Pharmacia, Upsala, Sweden.

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RED BLOOD CELL DIAMETERS IN WET AND DRY PREPARATIONS

By G. LARSEN

From the Med. Dept., Halden komm. sykehus, Halden, Norway

(Received for publication Dec. 15, 1954)

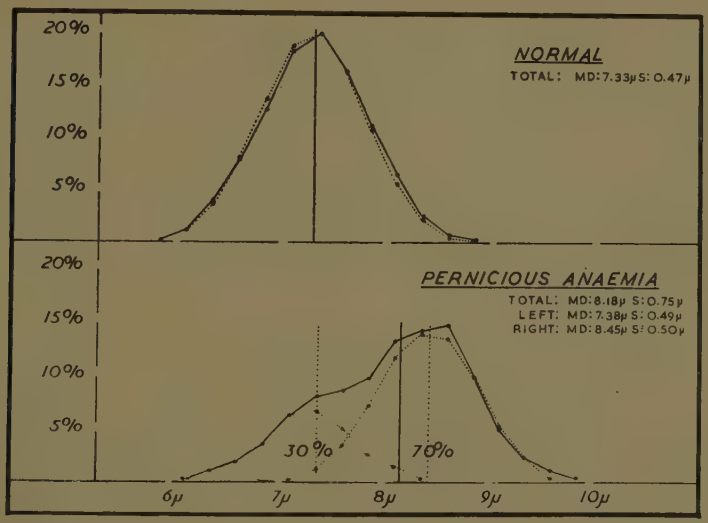
An increased degree of anisocytosis in untreated pernicious anaemia is an established fact. This increased anisocytosis is adequately demonstrated in diameter distribution curves. Usually these curves are prepared by measuring cells in the dried blood smear ("the dry method"). Measuring an adequate number of cells suspended in plasma or saline ("the wet method") presents technical difficulties. This method is therefore more often reserved for simple determination of the mean diameter.

It is generally held that the act of drying and staining the cells does not alter the cell size *per se*. However, most comparative studies between the dry and the wet method have been carried out on samples of normal blood, and such measurements are greatly influenced by numerous conditions. Slight differences as to optical equipment, the color of the light, the method used for measuring (whether by photography, direct micrometry, or otherwise) and other factors may play an important role. This makes direct comparison between methods uncertain. The question has been discussed in detail elsewhere (Larsen, 1948).

When red cells are measured in the dried state, distribution curves from normal persons conform with the normal distrib Corresponding curves from cases o nicious anemia are irregular, with a creased standard deviation. In different it may be shown that these irregular c are made up of at least two separate ponents, each normally distributed a its mean, with a standard deviation w normal limits (Baar & Lloyd 1943, L 1948, Mogensen 1938, Tötterman 1 One of these components has all the ch teristics of normal cells, while the other ponent consists of macrocytes with a diameter 0.75—1.00 micron above n values (Fig. 1).

Recently the author has shown that pathological cells are thinner than n cells in the dried state. In fact, the v of these dried pathological cells is ficantly below the volume of dried n cells (Larsen 1952). This in contrast to is found in fresh blood, where samples taining macrocytes usually, but not al have an increased mean corpuscular vo

To explain this discrepancy, it be necessary to make comparative studi the cell diameters, where normal and p logical blood cells are measured in the w well as in the dry state. The adhesio



Red cell diameter distribution curves. In normals the curve conforms with the normal ion. (Stippled line.) In cases of pernicious anemia the curves may be broken up into two normally distributed components, one of which has a normal mean.

ell surface and the glass slide may play an important role, and it will be of especial interest to examine size in the wet state using glassware non-wettable surface.

must be taken to ensure identical onditions for the examination of the samples. The results are best d by studying the diameter distribu- ves (the Price-Jones curves).

METHODS

was obtained by finger puncture. The rops were discarded and the following ere used alternately for preparation of ears and for blood cell suspension in

ation of slides for "wet preparations". er slips, 0.2 mm thick, were cemented rdinary slide 10 mm apart. When a third was placed on top, a space ensued s filled with blood cell suspension and sealed off with paraffin wax. The slides were examined within 5 minutes of preparation.

For "the silicon preparations" slides so prepared, together with cover slips, test tubes and all other glassware were treated with silicon (General Electric Dri Film, former SC-87) ensuring a non-wettable surface throughout.

For *the "wet preparations"* untreated glassware was used.

For *"the dry preparations"* blood smears were made in the usual way. The slides remained unstained, but were fixed by immersion in methyl alcohol. After drying, cover slips were cemented on the slide as described, and the space was filled with plasma.

Thus in the wet as well as in the dry preparations, the light from the microscope lamp passed through the glass slide, the cells, the plasma layer and the cover slip. Dried, unstained blood cells have too little contrast to photograph well by ordinary methods. All preparations were therefore photographed in a phase contrast microscope with enlargement 1000 ×, the cover slip used being sufficiently thin to allow the use of an oil immersion objective.

Table I. *Characteristics of red cell diameter distribution curves.*

									Analysis of pathological curves				
			Silicone prep.		Wet prep.		Dry prep.		Normal comp.			Macrocytic co	
Case No.	R.B.C. mill.	M.C.V cubμ	Mean. Diam. μ	Stand. Dev. μ	Mean. Diam. μ	Stand. Dev. μ	Mean. Diam. μ	Stand. Dev. μ	Mean. Diam. μ	Stand. Dev. μ	%	Mean. Diam. μ	Stand. Dev. μ
1	5.10	90	6.35	0.50	6.60	0.47	7.40	0.49					
2	4.60	86	6.39	0.53	6.59	0.53	7.17	0.51					
3	4.80	77	6.87	0.52	7.21	0.51	7.50	0.48					
4	4.81	91	6.34	0.51	6.75	0.53	7.18	0.54					
5	5.56	81	6.53	0.51	6.79	0.51	7.52	0.50					
Mean			6.54	0.51	6.79	0.51	7.31	0.50					
6	3.63	110	6.99	0.64	7.16	0.68	7.90	0.72	7.16	0.51	30	8.19	0.51
7	2.17	120	7.47	0.56	7.67	0.61	8.02	0.97	6.73	0.54	15	9.19	0.48
8	3.30	122	7.58	0.56	7.69	0.61	8.23	0.69	7.65	0.53	45	8.61	0.46
9	2.64	128	7.14	0.56	7.30	0.74	8.52	0.69	8.25	0.44	66	9.19	0.46
10	2.89	116	7.30	0.55	7.46	0.66	8.17	0.77	7.45	0.46	55	8.55	0.48
Mean			7.30	0.57	7.46	0.66	8.17	0.77	7.45	0.50		8.55	0.48

Details of the measuring method, as well as a description of the method used for the mathematical analysis of the distribution curves, has been reported previously (Larsen 1948).

RESULTS

For this study 5 normal individuals and 5 patients with untreated pernicious anemia were examined. The results are given in Tables I—II and in Figs. 2—3.

In normals (cases 1—5) the means of the wet preparations are larger than the means of the silicon preparations, and the means of the dry preparations even more so. But the standard deviation and the shape of the curve remain unchanged, and all the curves conform satisfactorily with the normal distribution as revealed by χ^2-analysis (Table II). The only difference between the different preparations is that the curves of the dry preparation are "shifted to the right".

One may conclude that in normal cases the preparation of a blood film affects all the cells in a like manner: During sprea and drying the cells are flattened out, increasing their diameter by about 12 cent as compared with cells placed non-wettable surface. Cells examined i wet state on ordinary glass flatten o a lesser, and to a less predictable de This is probably due to adhesion betwee cell surface and the glass. But all the are affected in a like manner, and ther their relative size remains the same, an shape of the distribution curve is changed.

In pernicious anemia (cases 6—10) results are different:

In the "silicon preparations" the diameter, as well as the standard devi is increased above normal values. Bu curves are all symmetrical, and the χ^2 lysis reveals a conformity with the n distribution as satisfactory as for the sa from normal persons (Table II).

e II. *Observed red cell diameter distribution curves compared with theoretical distribution. χ^2-analysis.*

Observed curves compared with normal distribution.									Pathol. curves compared with theoretical Compos. curves.		
„Silicon prep."			„Wet prep."			„Dry prep."					
χ^2	D. o. fr.	P	χ^2	D. o. fr.	P	χ^2	D. o. fr.	P	χ^2	D. o. fr.	P
3.889	8	0.80	9.880	8	0.20	7.023	8	0.50			
1.156	8	0.99	1.897	8	0.98	4.997	8	0.70			
3.604	8	0.80	4.516	8	0.80	2.771	8	0.90			
3.953	8	0.80	7.147	8	0.30	2.806	8	0.90			
3.362	8	0.80	5.645	8	0.50	3.891	8	0.80			
5.966			29.095			21.488					
4.377	8	0.80	7.258	10	0.70	6.483	10	0.70	5.995	8	0.80
1.497	8	0.99	5.189	10	0.80	14.553	10	0.10	3.541	8	0.80
3.573	8	0.80	7.814	10	0.50	17.642	10	0.05	4.490	8	0.70
1.401	8	0.99	4.123	10	0.90	18.301	10	0.05	6.238	8	0.70
3.673	8	0.80	6.374	10	0.70	16.492	10	0.05	4.963	8	0.70
4.521			30.758			73.471			23.277		

correspond well with the increased rpuscular volume found in these and seem to indicate that the ery- in pernicious anemia really are ıan normal cells and have an in- legree of anisocytosis.

dry preparations" all present distri- ırves generally thought to be typical s of untreated pernicious anemia: n diameter is increased by ca. 12 as in the normal cases. The standard is greatly increased in all cases, curves are all skew and platycurtic. out of 5 curves differ significantly normal distribution when examined alysis. Fig. 3 shows that "the wet ons" have distribution curves of a termediate between the two other

istribution curves from the patho- ıses were all analysed according to ıod described elsewhere (Larsen 1948). Neither the curves from the silicon preparations, nor the curves from the wet preparations could be broken up into distinct components, and all these curves fulfilled the criteria for a homogeneous population. In contrast to these findings, all 5 curves from the dry preparations could be broken up in at least two components, one of which had a normal mean which did not differ statistically from the means of the dry preparations of normal persons. The mean diameter of the pathological component averaged 8.55 microns against the normal mean 7.31 microns. Each of the two components had a standard deviation within normal limits, and the shape of the pathological curve was thus determined by the relative strength of the two separate components. When the observed curves were compared with theoretical curves composed of two normally distributed populations of

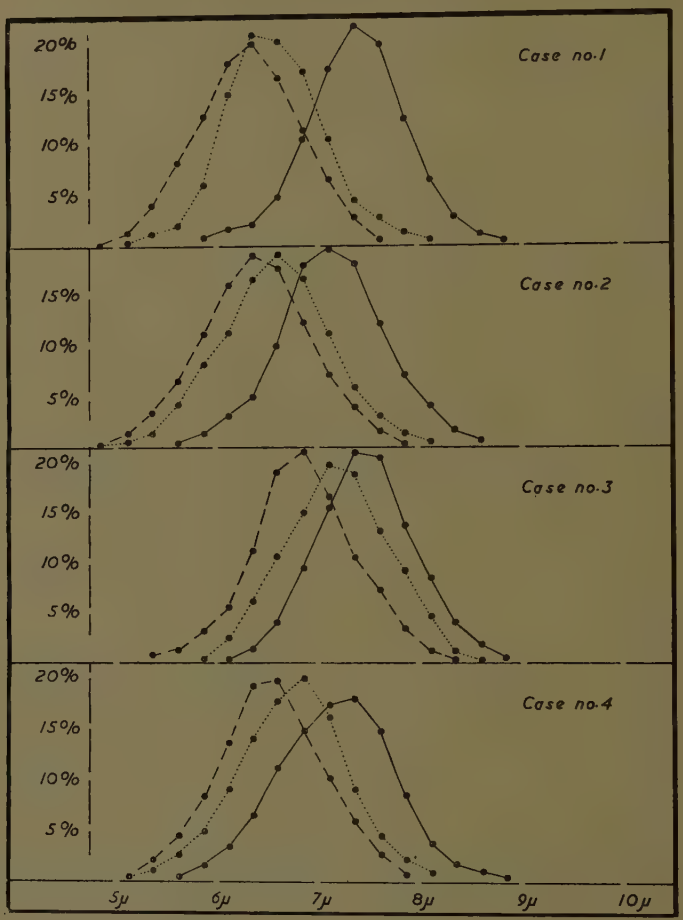


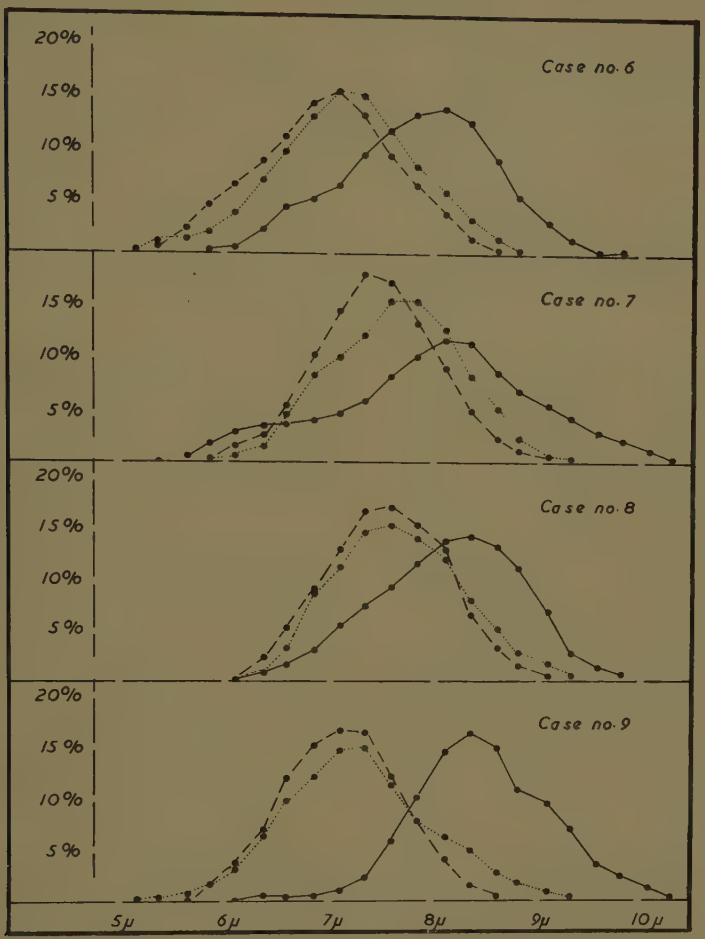
Fig. 2. Red cell diameter distribution curves from normal persons. Broken line: "Silicone prep tion". Stippled line: "Wet preparation". Unbroken line: "Dry preparation".

the same strength, and with the same means as that revealed by the analysis, the χ^2-test became as satisfactory as in the normal cases (Table II).

DISCUSSION

Macrocytosis as revealed by an increased mean diameter is a typical finding in cases of pernicious and related anemias. The mean corpuscular volume is usually increased too, but to a lesser degree and not corresponding to the increase in the mean diameter this is measured in dry preparations present investigation offers an expla of this conflicting evidence:

In normal blood all red cells are n cytes which behave in a like manner spread onto a glass slide and dried. cells flatten out so that the diameter inc by about 12 per cent, but all the ce affected to a like degree, and therefo



Red cell diameter distribution curves from cases of untreated pernicious anemia. Broken line: one preparation". Stippled line: "Wet preparation". Unbroken line: "Dry preparation".

the distribution curve as well as the deviation remain the same.

treated pernicious anemia the peri- lood contains normocytes and macro- The latter are slightly larger than cells, and therefore the mean dia- the cells placed on a non-wettable is increased. The mean corpuscular s increased. But the difference from nals is not of such a magnitude that the presence of macrocytes may be ascertained.

When blood from these patients is spread onto a glass slide and dried, the macrocytes behave differently from normal cells: They flatten out more than do normal cells, their diameter increasing about 17 per cent against the normal increase of 12 per cent. This causes the distribution curves to alter their shape. The curves no longer conform with

the normal distribution and the heterogeneity of the population becomes evident. The author has previously shown that these dried macrocytes have a smaller volume than dried normal cells (Larsen 1952). As their volume in the wet state is greater than normal cells, it follows that they shrink more during the act of drying. It seems therefore reasonable to assume that the macrocytes have a greater content of water than normocytes, and that the forces of adhesion between the cell surface of the macrocyte and the glass are greater than between the cell surface of the normocyte and the glass.

It may be argued that the abnormal distribution curves found in dry preparations from cases of pernicious anemia are artefacts brought about by the preparation of the slides. But it is a reproducible artefact and, so far, study of the diameter distribution curves in dry preparations affords the only means for estimating the amount of macrocytes in a blood sample.

Finally, it may be pointed out that wet preparations on ordinary glass slides present the abnormalities found in the dried blood smear to a lesser, and probably unpredictable degree. Examination of the cell size in the wet state ought therefore always to be carried out using glassware with a non-wettable surface.

SUMMARY

Red blood cell diameter distribution cu from cases of untreated pernicious an differ from normal curves when meas ments are carried out on dried cells. characteristic and abnormal curves can be demonstrated when blood cells suspe in plasma are placed on a non-wettable face and measured.

Evidence is presented suggesting that abnormal diameter distribution curves in preparations, characteristic of pernicious related anemias, are caused by the pres of macrocytes which behave differently normocytes when the cells are placed glass slide and dried. The differenc assumed to be due to different degree o hesion and differences in water conten tween the macrocytes and the normoc

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PARISON OF THE PROTHROMBIN-PROCONVERTIN ERMINATIONS (OWREN-AAS) WITH "PROTHROMBIN-INDEX" OBTAINED WITH THE LARSEN-PLUM PROTHROMBIN TIME METHOD[1]

By ERLING LUND, TORBEN GEILL, EBBE SØNDERGAARD AND HENRIK DAM

he Department of Medicine, De Gamles By (Old Peoples Town), and Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen, Denmark

(Received for publication Dec. 6, 1954)

increasing use of the Owren-Aas for determining prothrombin-pro- n values has rendered it desirable to this method with the prothrombin thod of Larsen & Plum.

ugh some investigators (Astrup, Mül- Rud Hansen 1951 and Begtrup) have already reported such studies found it of interest to publish our partly because they comprise a larger of determinations than hitherto re- nd partly because we have used a iable technique for the Larsen-Plum

ermore, we wish to introduce certain view which have hitherto not been fficient consideration.

ATERIALS AND METHODS

ethod described by Owren & Aas (1951) d by Aas (1952) was used for determina- he combined effect of prothrombin and tin (the so-called PP-value).

ndex" was calculated as the ratio between rombin time of a normal individual and he patient. The prothrombin time was d by the method of Larsen & Plum ing human brain thromboplastin prepared to Dam & Glavind (1938).

Hypoprothrombinemia was induced by dicoumarol and dicoumarol-like anticoagulants.

With each method 765 determinations were performed (on 63 patients).

RESULTS AND DISCUSSION

The results are shown in Figures 1—5.

The diagram (Fig. 1) shows a considerable scattering of the results obtained with the two methods. A small number of points

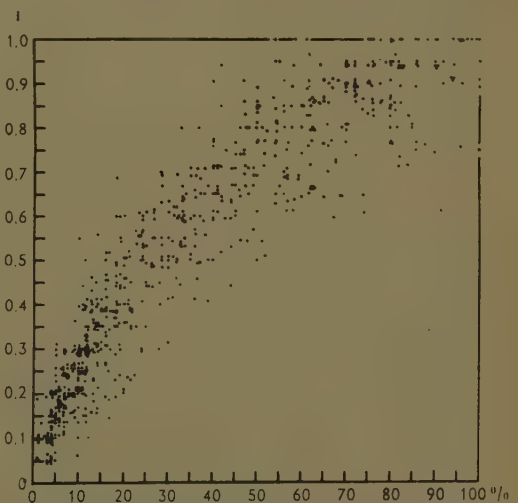


Fig. 1. Relation between "Index" calculated from Larsen-Plum prothrombin times and the Owren—Aas prothrombin-proconvertin value (PP-value). *Abscissa:* prothrombin-proconvertin concentration (PP-value) in per cent of normal. *Ordinate:* "Index".

rted by a grant to Dr. Torben Geill from h State Research Foundation.

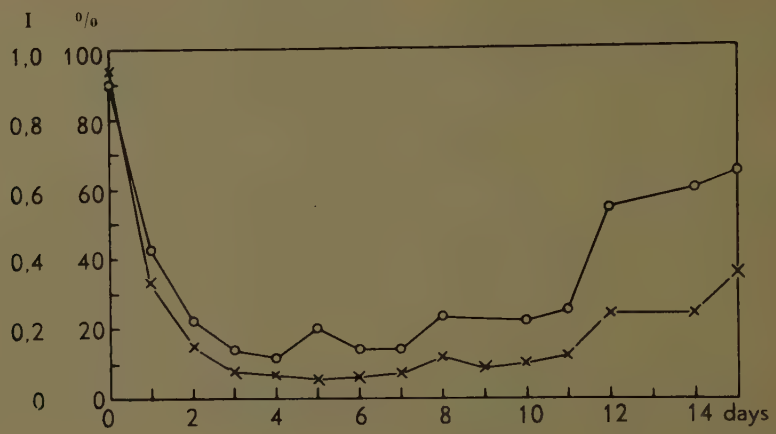
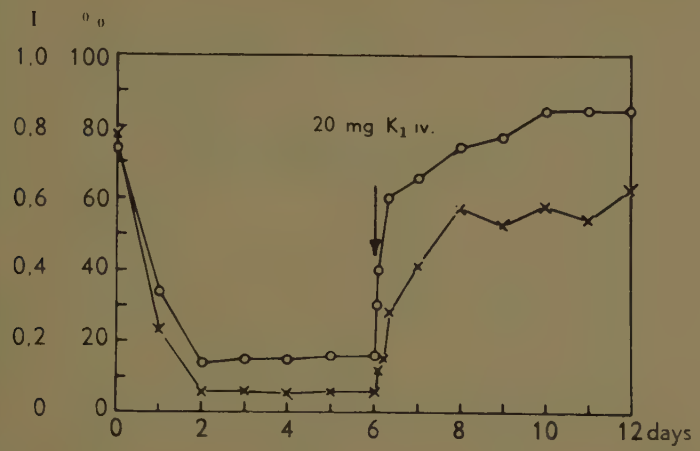


Fig. 2. "Index" calculated from Larsen—Plum prothrombin times o-o-o- and Owren—Aas prothr bin-proconvertin value x-x-x- during anticoagulant therapy. *Abscissa:* days after single dose of mg 2-p-chlorphenyl-1.3-indanedione. *Ordinate:* "Index". Prothrombin-proconvertin value (P value) in per cent of normal.

with PP-values higher than 100 per cent or with "Index" of more than 1.0 are not shown in the figure. The question now arises: which of the two methods is of the greatest practical value in determining the coagulability of a patient's blood. The Owren-Aas method results in a figure which is proportional to the combined activity of the two components: prothrombin and proconvertin, while other factors are held constant. The Larsen-Plum prothrombin time is a complex function ($y = f(x_1\ x_2\ x_3\ \ldots)$) of the combined action of prothrombin and proconvertin plus other factors involved in the coagulation process, while only thromboplastin and Ca^{++} are held constant. Therefore, this method will reflect not only variations in prothrombin and proconvertin but also variations in the level of other coagulation factors present in the blood sample under investigation.

It is, therefore, of importance to k whether variations in such other factors have occurred after the blood sample drawn. If this has been the case, the p obtained by the Larsen-Plum method ca be referred to characteristics of the pati blood and thus will be of very little v In our experiments, however, the time tween drawing of the blood and the d mination was about 1 hour. Accordi Larsen (1952) only negligible variatio the Larsen-Plum prothrombin time occur within 2 hours (in the direction shortening of the prothrombin time). I practical reasons (such as long transp tion *etc.*), the determination cannot be ried out within such a short time, mo less considerable errors may be introd (initially due to activation of procon (Rapaport, Aas & Owren 1954) an disappearance of proaccelerin). Au



Index" calculated from Larsen—Plum prothrombin times o-o-o- and Owren—Aas prothrom-nvertin value x-x-x- during anticoagulant and vitamin K_1 therapy. *Abscissa:* days after se of 20 mg 2-p-chlorphenyl-1,3-indanedione. *Ordinate:* "Index". Prothrombin value (PP-value) in per cent of normal.

be considered is whether the dilu- blood (0.1 in 0.375) in the Larsen- thod influences the activity of the coagulation factors to different whereby the coagulation time ("pro- time") observed would give an ed picture of the state of coagula- ne original undiluted blood. Should be the case, then the prothrombin the "Index") will provide a better ation of the actual state of coagula- the patient's blood than a specific tion of the combined activity of rs. If the "Index" is to be used tion with dicoumarol therapy, then s to be decided which interval of is optimal. As regards the PP- most investigators find that the 10—25 or 10—30 per cent is y and recommend the method as or the control of anticoagulant therapy (Bjerkelund 1953, Lund 1953 a, b, Owren 1954, Warburg 1954). However, Begtrup (1954 a, b) has suggested the possibility that this interval may be reduced further, *e. g.* to values between 5 and 10 per cent without any appreciable risk.

It is obvious that the figure called "Index", *viz.* the ratio between the prothrombin time of the supposed normal individual and that of the patient is not and *cannot be proportional* to the concentration or activity of any or a combination of some of the factors taking part in the clotting process since the function $y = f(x_1, x_2, x_3 \ldots)$ is not linear. The Larsen-Plum technique does not in itself include the calculation of an "Index". The way in which prothrombin times determined by this method were used for calculation of "prothrombin activity" was by means of a standard curve based on prothrombin values determined by the method

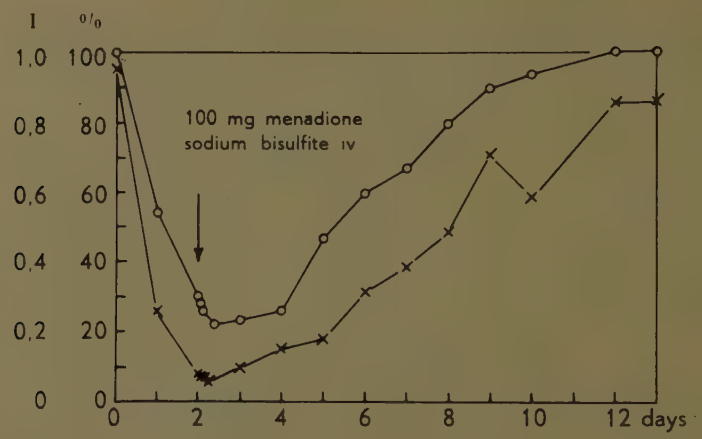


Fig. 4. "Index" calculated from Larsen—Plum prothrombin times o-o-o- and Owren—Aas proth bin-proconvertin value x-x-x- during anticoagulant and menadione sodium bisulfite treatment. *scissa:* days after single dose of 20 mg 2-p-chlorphenyl-1,3-indanedione. *Ordinate:* "Index". thrombin-proconvertin value (PP-value) in per cent of normal.

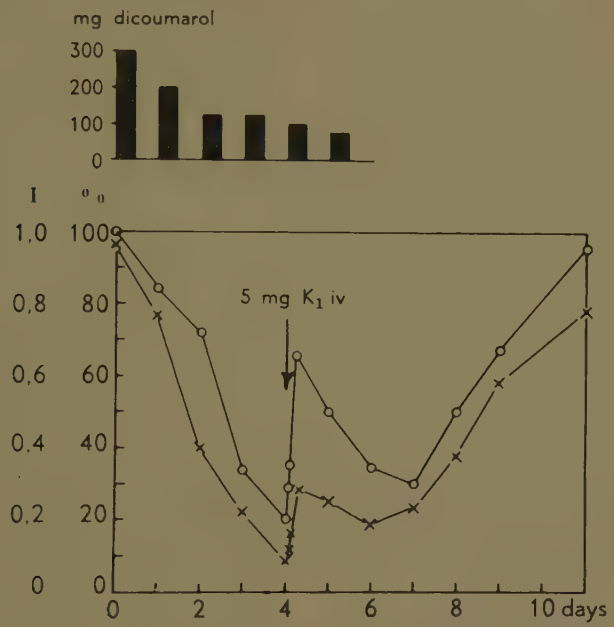
of Thordarson (1941) as described in detail by Larsen (1952), who also points out the shortcomings of the use of "Indices". Nevertheless, within a certain range of low prothrombin activities, the "Index" has been used by many authors as a measure of the state of coagulability of a given sample of blood with constant thromboplastin and Ca^{++}. "Indices" can only be compared when the corresponding prothrombin times are determined with one and the same thromboplastin preparation and exactly one and the same prothrombin time method.

Even with this qualification, the "Index" is of little value in the range above 50 per cent of the normal prothrombin-proconvertin activity.

As already mentioned, comparison between prothrombin-proconvertin values (Owren-Aas) and prothrombin times (Larsen-Plum) or "Indices" calculated from the latter has been undertaken by other authors, *e. g.* Astrup, Müllertz & Rud sen (1951) and Begtrup (1954 a, b) both of these studies dried rabbit extracted with acetone has been us thromboplastin. According to Larsen (this is much less satisfactory than brain thromboplastin prepared accord Dam & Glavind (1938) and stored i dried frozen state. Astrup *et al.* found that their normal prothrombin varied from 14 to 32 sec. Such var have never been found in Larsen's or in our experiments with human thr plastin.

Astrup *et al.* (1951) have stated matical equations between the prothr concentrations and prothrombin times. equations do not include the influence many variables involved. It would a

[1] Begtrup refers to prothrombin, but ap means results obtained with the prothrom convertin method.



"Index" calculated from Larsen—Plum prothrombin times o-o-o- and Owren—Aas prothrom-onvertin value x-x-x- during dicoumarol and vitamin K1 therapy. *Abscissa:* days after first dicoumarol. *Ordinate:* "Index". Prothrombin-proconvertin value (PP-value) in per cent of normal.

icult to account for the influence of variables in a mathematical equa-

sidering Begtrup's results (1954a, b, which are obtained with a smaller of determinations (237) than ours appears that there would be better it with our results if Begtrup's alue for "Index" were put at 120 f 100. The reason for this is not explain. However, the determina- Indices" in cases treated with dicou- ry in an unexplicable manner in s experiments (1954 a, b, Figs. 2

experiments carried out by us –5) show much less variation.

SUMMARY

From 63 patients treated with dicoumarol anticoagulants, 765 blood samples have been examined by the prothrombin-proconvertin method of Owren-Aas and by the prothrombin time method of Larsen-Plum (with human non-dried brain thromboplastin).

"Indices" have been calculated on the basis of the latter method and plotted against the PP-values.

The scattering of the points representing indices is not necessarily due to errors inherent in the Larsen-Plum method, but may reflect individual variations in coagulation factors which are kept constant in the Owren-Aas method.

The two methods have been used for daily examination through 10—15 days or more of patients treated with dicoumarol-anticoagulants with and without vitamin K. The results show less variation of the "Index" than found by authors who have worked with acetone extracted dried rabbit brain.

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GNIFICANCE OF AZOTEMIA IN GASTRO-INTESTINAL HEMORRHAGES

By H. N. HAUGEN

From Ullevål Hospital, Department Krohgstøtten, Oslo, Norway

(Received for publication Dec. 22, 1954)

currence of azotemia in gastro-hemorrhages is well known, but ly understood. Differences in ll exist regarding the part played estion and absorption of the great f blood, shock, *et cetera*. As no the plasma "creatinine" concentra- appeared, the purpose of the pre- is to report the results of a study "creatinine" values in gastro-emorrhages. It is hoped that the reatinine" study might give some n regarding the pathogenesis of ia.

TERIAL AND METHODS

a and plasma "creatinine" were studied nts suffering from gastro-intestinal . There were 16 men and 11 women, 3 years. Eleven patients suffered from uodenal ulcer, the diagnosis being con- -ray, one had cancer of the stomach, s of the liver and esophageal varices, patients no cause was found. Blood plasma "creatinine" were examined on admission and at regular intervals stay in hospital. All blood samples, rst one, were drawn in the postabsorp- the morning.

ea was determined by Van Slyke's method, plasma "creatinine" by the ate reaction (saturated picric acid and NaOH). Plasma protein was precipitated by the Brod & Sirota (1948) modification of the Folin & Wu method. The color was read after exactly 10 minutes in a Coleman Junior spectrophotometer (wave length 500 mμ) or in a Klett-Summerson colorimeter at 23° C.

The upper normal limit of blood urea depends upon the protein consumption and the diuresis. In this study 40 mg/100 ml was regarded as the upper limit. In normals we never observed plasma "creatinine" values above 1.3 mg/100 ml.

No patient showed proteinuria, hypertension or other signs of renal disease.

RESULTS

According to the ratio blood urea-plasma "creatinine" values, the patients may be grouped in the following way:

Group I: Four patients with normal blood urea and plasma "creatinine" values. Table I shows an example: Patient O. F., 60 years old, was admitted for melena. He was in good condition, with hemoglobin 110 per cent (Haldane), red blood cells 5.5 mill./mm^3 of blood. Blood urea was 20 mg/100 ml, plasma "creatinine" 1.03 mg/100 ml. The stools gave prompt reaction on blood. Hemoglobin fell to 94 per cent after a few days, red blood cells to 4.7 mill., whereas blood urea and plasma "creatinine" were unaltered.

The three other patients in this group behaved in exactly the same manner. Common to all was good condition, only insignificant

Table I.

Date	Blood urea (mg/100 ml)	„Creatinine" (mg/100 ml of plasma)	Hemoglobin (per cent)	Red blood cells (mill.s/mm³)	Blood pressure (mm of mercury)	Benzidine reaction in feces
24/4 1954	20	1.03	110	5.5	120/80	++++
28/4 1954	22	1.06	94	4.7		÷
3/5 1954	25	1.00	96	5.2		÷

Table II.

Date	Blood urea (mg/100 ml)	„Creatinine" (mg/100 ml of plasma)	Hemoglobin (per cent)	Red blood cells (mill.s/mm³)	Blood pressure (mm of mercury)	Benzidine reaction in feces
22/3 1954	70	1.06	70	3.50	140/80	++++
25/3 1954	32	1.08	66	3.46	125/75	+++
5/4 1954	32	1.11	70	4.20		+
4/5 1954	26	1.11	89	4.50		÷

reduction of the blood values, normal blood urea and plasma "creatinine".

Group II: Eighteen patients had raised blood urea, but normal plasma "creatinine" values. Patient C. H. (Table II), 64 years old, was on admission a little pale, but in good condition. Hemoglobin was 70 per cent, red blood cells 3.5 mill./mm³. The benzidine reaction in feces was ++++. Blood urea was 70 mg/100 ml, plasma "creatinine" 1.06 mg/100 ml. No further depression of hemoglobin occurred. Within days blood urea became normal, 32 mg ml, whereas plasma "creatinine" was changed. The benzidine reaction negative. Blood urea and plasma "creat values remained normal on subs examinations.

Table III.

Date	Blood urea (mg/100 ml)	„Creatinine" (mg/100 ml of plasma)	Hemoglobin (per cent)	Red blood cells (mill.s/mm³)	Blood pressure (mm of mercury)	
8/9 1953	71	1.65	92	4.6	unmeasurable	÷
9/9 1953	120	1.77	76		115/80	+
10/9 1953	134	1.31	64	3.2	110/75	
11/9 1953	63	1.17	68		110/70	
12/9 1953	44	0.98	73	3.5	120/80	÷
13/9 1953	38	1.00	72			
14/9 1953	35	1.00			110/75	

The other patients in this group b in the same way. Common to all wa condition, moderate anemia (no hemo value below 48 per cent), raised bloo (highest value 110 mg/100 ml), but plasma "creatinine" concentration. In patients in this group plasma "creat concentration remained unchanged the stay in hospital.

Group III: Five patients in who blood urea as well as plasma "creat values were raised. Patient A. F. III), 46 years old, was admitted in

ion, exhausted, obviously suffering
s, without measurable blood pres-
oglobin was 92 per cent, red blood
ill./mm³, but the values fell within
f days to 64 per cent and 3.2 mill.
repeated blood transfusions. His
e tarry and the benzidine reaction
+. Blood urea was on admission
ml and increased to 134 mg/100
upon it in the course of a week
mal values. Plasma "creatinine"
nission moderately increased, 1.65
, on the next day 1.77 mg/100 ml,
ally fell to about 1 mg/100 ml and remained at this level. His condition improved after some days and he felt well.

All patients in this group behaved in this same way. On admission they were in poor condition, suffering from shock, showing reduced hemoglobin concentration, significantly raised blood urea and plasma "creatinine" values. After treatment, blood urea and plasma "creatinine" fell to normal levels.

No one can estimate how much blood these patients lost into the intestines. Blood transfusions were given to those who showed low hemoglobin values or obviously were suffering from shock. No patient in group I needed blood transfusion. In group II, 11 out of 18 patients were given blood transfusions, on an average 1.4 liter per patient. All patients in group III needed and received transfusions, on the average 3.7 liters per patient. Using 100 per cent of hemoglobin as normal value, the average reduction of hemoglobin in group I was 16 per cent, in group II 33 per cent, in group III 41 per cent. Figure I shows good agreement between reduction of hemoglobin concentration and increment of blood urea. The patients with the lowest hemoglobin values show the highest blood urea levels.

DISCUSSION

This study confirms previous reports that azotemia frequently occurs in patients suffering from gastro-intestinal hemorrhages. Twenty-three out of 27 patients studied showed raised blood urea values, which agrees well with Schrumpf's (1944) observation (azotemia in 43 out of 55 patients). Contrary to this, plasma "creatinine" was increased in only 5 patients, while the remaining 22 patients showed not only normal

values, but values which during the stay in hospital were completely unaltered.

Attention has been drawn to different causes of the azotemia. Black, Powell & Smith (1941) found the inulin clearance considerably reduced while the blood urea concentration was rising, while on the other hand Stevens, Schiff, Lublin & Garber (1940) maintained that the elevation of the blood urea nitrogen which follows hematemesis in the absence of shock is not due to impairment of kidney function. Schrumpf concluded that the initial azotemia is caused by subnutrition; he found azotemia also in ulcer patients without hemorrhage (his patients were treated with the old-fashioned ulcer diet which for the first 8—10 days is calorically insufficient). He further pointed out that the intestinal digestion of blood may to a certain degree participate in the production of azotemia, whereas hemodynamic factors are without significance. He was unable to demonstrate reduction of renal function as a cause of the azotemia.

Borst (1938) maintained that the azotemia depends upon raised urea production through the intestinal digestion of blood. On a diet calorically insufficient, destruction of body protein will occur which will give additional urea production. If the patient, on account of recurrent hemorrhages, becomes exhausted, a further rise in urea concentration will ensue. Borst's patients were given a low caloric diet for the first days after admission to hospital .

All our patients received a soft ulcer diet containing about 2000 calories and 70 grams of protein a day. They were also allowed a liberal amount of fluid. All had a urinary output of more than 700 ml/24 hours. Undernutrition or lowered diuresis as causes azotemia are therefore excluded. On possibilities may explain the raised urea, *viz.* 1) raised urea production intestinal digestion of blood, 2) dep renal function.

A shown by Addis, Barrett, Poo & (1947), blood urea concentration pendent upon protein consumption. protein supply will increase blood urea As blood contains much protein (one blood contains about 100 grams of pr it is not surprising that intestinal bloo duces azotemia. If the patient is give calories as in the old-fashioned ulcer the azotemia may under certain circums be lower than if a high caloric, high p diet is given, as urea from the di dietary protein will add to urea from di blood. Certainly a great fraction of the will be digested and resorbed and thu duce urea. It is to be expected that th severe hemorrhages will give rise highest blood urea values. Figure firms this, as it will be seen that the h blood urea values are found in those p having lost the largest amounts of blo

However, renal function is also w doubt of importance for the developm azotemia. Many patients suffering bleeding ulcers are exhausted, pale; pressure is low, and in these patien azotemia may be due to a transitory functional depression. The study of "creatinine" values confirms this view plasma "creatinine" concentration d upon two factors, *viz.* the amount of tinine" produced throughout 24 hou the amount excreted through the ki The production being constant, the

ıe" concentration depends only
al function. "Creatinine" in digested
without importance, as one liter of
ıtains only 10—15 mgms of "crea-
In 22 of the 27 patients studied a
lasma "creatinine" level was found,
level was unaltered during their
ospital. This shows that the renal
was completely normal. However,
nts a high plasma "creatinine" con-
ı was found shortly after admission,
er on the value became normal.
tients were all exhausted, suffering
ck, with low blood pressure, and
ded repeated blood transfusions.
ease of plasma "creatinine" signified
ed renal function. The depression
temporary, and after treatment
'creatinine" values fell to normal
n these 5 patients the temporary
ression participated in the develop-
azotemia, in three only moderately,
o such an extent that blood urea
to 134 and 183 mg/100 ml and
reatinine" to 1.77 and 1.45 mg/100
ctively. Azotemia of this degree is
found even on a very high protein
ion as long as renal function is

CONCLUSION

zotemia in gastro-intestinal hemor-
due mainly to intestinal digestion
orption of blood. In exhausted
he renal function is depressed; this
further rise of blood urea. The depression of renal function can most easily be diagnosed through the demonstration of an increased plasma "creatinine" concentration.

SUMMARY

Blood urea and plasma "creatinine" values were studied in 27 patients suffering from gastro-intestinal hemorrhages. Four patients showed normal blood urea and plasma "creatinine" concentration, 18 raised blood urea and normal plasma "creatinine" values, while 5 showed raised blood urea and plasma "creatinine" levels. The significance of these relationships is discussed.

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LEAD INTOXICATION: I. THE EFFECT OF LEAD ON THE *IN VITRO* BIOSYNTHESIS OF HEME AND FR ERYTHROCYTE PORPHYRINS

By L. ERIKSEN

From the Institute of Physiology, University of Oslo, Norway

(Received publication Oct. 25, 1954)

It has long been known that lead causes marked changes in blood and in the composition and amount of excreted porphyrins.

Aub, Smith & Raznikoff (1924) ascribed the blood changes to increased destruction of the red corpuscles. Rimington (1938) suggested that lead inhibits hemoglobin synthesis by preventing the incorporation of iron into the protoporphyrin nucleus, resulting in an increased porphyrin excretion.

Kench, Gillam & Lane (1942) in a study of blood and urinary porphyrins in lead workers, came to the conclusion that the physiological action of small amounts of lead cannot be explained in terms of a partial prevention of iron-porphyrin complex formation, but most probably is due to a restricted cellular activity resulting in a diminished formation of protoporphyrin.

Shemin, London & Rittenberg (1948) showed that duck erythrocytes were able to synthesize heme when incubated *in vitro*. London, Shemin & Rittenberg (1950) showed that also immature non-nucleated mammalien erythrocytes were able to synthesize heme *in vitro*.

The present study was undertaken to obtain more information on the effect of lead on porphyrin and heme biosynthesis, which is necessary for a basic study of the problems connected with the porphyrin in lead workers intoxicated with lead.

MATERIALS

The blood used was taken from normal ducks and from normal rabbits, 1—2 years and 2.8—3.4 kg in weight, made anemic by subcutaneous injections of 25 mg phenylhydr for 5 days and exsanguinated on the sixth d

In all cases the blood was collected into 0.9 per cent NaCl containing 10 mg heparin.

The cells were centrifuged down, washed 0.9 per cent NaCl, suspended in twice the v of the sodium chloride solution, and used i incubation experiments.

Carboxyl-labeled sodium acetate was obt from Nyegaard & Co. Its activity was 1 mc/M Solutions containing 2 μc per ml were mad and kept in the refrigerator throughout the e ments. 15.9 ml of a ferric ammonium citrate tion containing 7.9 mg Fe elemnt was obt from the Atomic Centre, Harwell, Englan activity was 898.5 μc/Mmol measured as (May 8, 1953) and with an almost equal a of Fe^{55}. All other chemicals used were of grade.

METHODS

Incubation: 15 ml cell suspension was in 100 ml Erlenmeyer flasks, plugged with stoppers and incubated at 37.5° C with a s rate of 100 shakes/min. An isotonic solut glycine was added to each flask to give a concentration of 2 mg glycine/ml cells.

In the experiments with radioactivity $CH_3C^{14}OONa$ was added just prior to t

When both the incorporation of radio-
c acid and iron were to be measured,
ne $Fe^{55/59}$ solution was added together
adioactive acetate.
vas prepared according to Ansson &
29), washed repeatedly with 0.2 N HCl,
NaOH pellets overnight in a vacuum
and dissolved in methanol. An excess
was added and the methanol evaporated
bath. On cooling, hemin precipitated
ivided state which adhered satisfactorily
ng cups, gave smooth plates, and which
converted to protoporphyrin dimethyl
ferrous sulfate and HCl gas. To ensure
known radioactive contamination was
his preparation, an experiment was per-
which part of the hemin was prepared
to Fischer (1941) and recrystallized
to Fischer & Pützer (1926), and the
ed according to the above procedure.
was plated and counted as described
seen from Table I, the difference in the
tivities of the two preparations was
counting error.

I. *Specific activity of heme pre-*
rding to Fischer (Nos. 1—4) and
Mirsky, recrystallized from metha-
nol/benzene (Nos. 5—6).

c/min/mg	Plate No.	c/min/mg
216.0	5	214.1
208.5	6	206,0
204.5	7	203.7
210.5	8	210.2
209.7		208.5

nin was dissolved in methanol, excess
fate was added and the solution gassed
HCl gas until the color changed from
violet. A small amount of chloroform
and then water was added until the
phase separated. The chloroform phase
d off, the water phases was washed a
imes with small amounts of chloroform
ed. The chloroform solution was washed
distilled water, once with 10 per cent
twice with distilled water, filtered on
chloroform-moistened filter paper, concentrated on a water bath and applied to a MgO colum grade II and chromatographed according to Nicholas (1951). The final chloroform solution contained no detectable iron. The solution was concentrated to a small volume on the water bath and the porphyrin dimethyl ester precipitated with an excess of petrol ether, washed a couple of times, suspended in 5 ml petrol ether and brought into special plating-cup holders, to be described elsewhere, and allowed to settle under a sligtly warm lamp which evaporated the petrol ether in about 2 hours.

The plate was removed from the holder and weighed. The amount of plated porphyrin ester was found by difference between the total weight and the weight of the plating cup.

The total activity was measured with a Tracer Lab. Superscaler using a TGC 2 Geiger tube for the counting of C^{14}.

When both radioactive carbon and iron were to be measured, the hemin, prepared as described, was divided in two parts. One was worked up as described above, and one was dissolved in hot concentrated nitric acid and kept on a slightly warm electric plate in the hood until the solution was clear and the color had changed to straw yellow. Then the solution was evaporated to almost dryness, 5 ml hot distilled water was added and the iron precipitated with an excess of concentrated ammonia. The ferric hydroxide was centrifuged down, washed twice with a dilute ammonium chloride solution, dissolved in a small amount of dilute HCl acid, and evaporated to almost dryness on a water bath. The ferric chloride was dissolved in 10 ml saturated ammonium oxalate, and the amount of iron measured in a Beckman spectrophotometer as the o-phenatroline iron-complex at 510 mmμ according to Fortune & Mellom (1938).

Then a known mixture of hemin iron and carrier iron was made and the iron plated electrolytically in an electrolysis apparatus, to be described elsewhere. Each plate always contained 3 mg of the iron mixture on a square *of 2.83 cm.* No self-absorption in the iron film could be measured at this thickness. The activity was measured with the Tracer lab.scaler, using a TGC3 Geiger tube for the counting of iron.

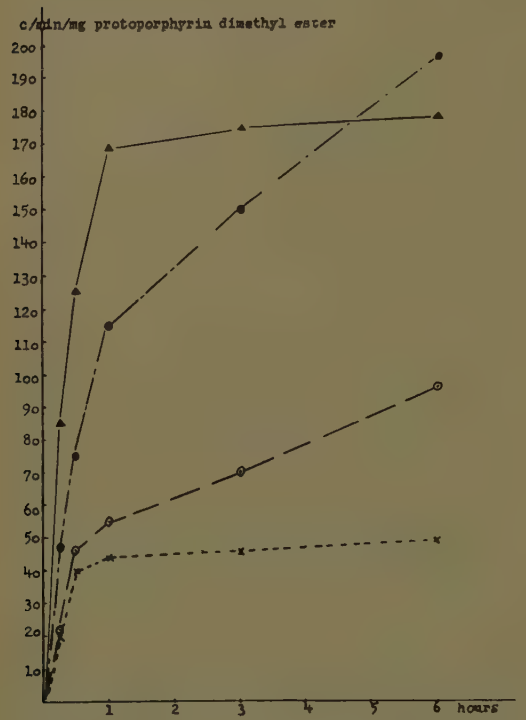

Fig. 1. The effect of Pb^{++} on the incorporation of carboxyl C^{14} of sodiumacetate in heme.
×·····× 10^{-4} Mol Pb^{++}, rabbit erythrocytes.
△——△ — Pb^{++}, rabbit erythrocytes.
⊙——⊙ 10^{-4} Mol Pb^{++}, duck erythrocytes.
●——● — Pb^{++}, duck erythrocytes.

The iron activity is given as counts per min. per mg hemin iron. The C^{14} activity is given as counts/min./mg protoporphyrin dimethyl ester corrected to zero thickness as described by Calvin, Heidelberger, Reid, Tolbert & Yankwich (1949). Sufficient counts were taken to give a standard error of less then ± 3 per cent.

Free erythrocyte porphyrins were extracted as described by Schwartz & Wikhoff (1952) and protoporphyrin measured according to Grinstein & Wintrobe (1948).

The free porphyrins were applied to small talc columns in approximately 1.0 N HCl solution. The columns were washed extensively with distilled water until free of chloride ions as judged by the $AgNO_3$ test. The porphyrins were than elu highly concentrated solution with 30 per ce per cent NH_3 in acetone, applied to paper and chromatographed as described by E (1953).

RESULTS

Effect of lead on the incorporation o carboxyl carbon of acetic acid into h

In each experiment 2 series of 5 in tion flasks were set up. To one series acetate was added to a final concentr of 10^{-4} Mol. Pb^{++}, to the other series added inactive sodium acetate to a final centration of 10^{-2} Mol. To all flasks added 2 μc carboxyllabeled acetate, the flasks incubated as described abov

The incubation flasks were taken o varying times. The cells were centri down, washed twice with 0.9 per cent and heme protoporphyrin prepared, and counted. The results of two t experiments are given in Fig. 1.

Effect of lead on the biosynthesis o erythrocyte porphyrins by duck erythro

In another investigation it has been s that there is no qualitative difference composition and biosynthesis of free ery cyte porphyrins in duck erythrocytes immature erythrocytes from phenylhydr rabbits (Eriksen 1954), and as the erythrocytes are able to synthesize heme a considerable speed for 6 hours, while erythrocytes are able to synthesize 1 hour only in the medium used (F duck erythrocytes were preferred in th sent experiment.

Two series of 5 flasks were incuba described. No radioactive markers wer in these experiments. The amount o

ns were measured at varying times. ows the results of a typical experi-

cases small amounts of 0.1 N HCl ble porphyrins were found to be pre- e spectral absorption in the so-called and was not that of coproporphyrin, ved a broad maximum at about 405 dicating a mixture of coproporphyrin to- or hematoporphyrin. The por- were brought back into ether and cted with 0.1 N HCl, and almost all orphyrin went into the HCl. Thus be a mixture of coproporphyrin and phyrin. The amounts of 0.1 N HCl n were very small and to get enough n for paper chromatography, all the Cl porphyrin fractions from the two ere collected in two portions, con- l on two small talc colums, eluted matographed on paper as described. er substances were used coproporII and mesoporphyrin which have dly supplied by Prof. Rimington. ts were found in each chromatogram nding to a dicarboxylic porphyrin racarboxylic porphyrin (Table II). ease in the ratio tetracarboxylic to ylic porphyrins was found in the ies as compared to the control

f lead on the incorporation of the carbon of acetate and of $Fe^{55/59}$ into

bation flasks were used. To each s added 2 μc $CH_3C^{14}OONa$ and $e^{55/59}$ solution. Lead acetate was four flasks to a final concentration

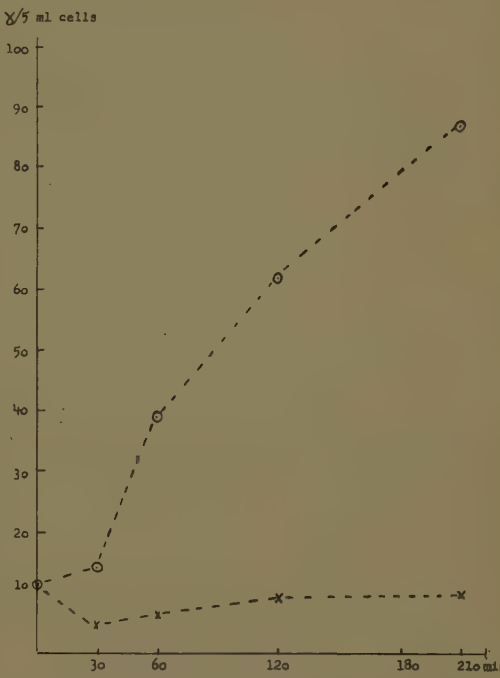

Fig. 2. The effect of Pb^{++} on the formation of free protoporphyrin in duck erythrocytes incubated in 0.9 per cent NaCl.

× ····· × 10^{-4} Mol Pb^{++}.
⊙——⊙ — Pb^{++}.

Table II. *The effect of Pb^{++} on the composition of 0.1 N HCl extractable porphyrins from duck erythrocytes incubated in 0.9 per cent NaCl.*

Carboxyl numbers	0.1 N HCl porphyrin	
	10^{-4} M. Pb^{++}	— Pb^{++}
2	(+)	+
4	(+)	+

of 10^{-4} Mol. $Pb.^{++}$. To the other four flasks inactive sodium acetate was added to a final concentration of 10^{-2} Mol. The flasks were incubated 1 hour, heme iron and protopor-

Table III. *Effect of Pb^{++} on the incorporation of carboxyl C^{14} of acetate and $Fe^{55/59}$ in heme.*

Medium	No.	c/min/mg prot.ester	c/min/mg Hemin Fe	sp. act. Fe sp. act. p.e.	Mean ratio
	1	200.4	5564	25.82	
	2	217.2	5614	27.76	
—Pb^{++}.....	3	208.0	5602	26.95	26.78
	4	204.2	5428	26.59	
	5	46.2	1316	28.49	
	6	40.3	1201	30.00	29.42
10^{-4} Mol.Pb^{++}	7	42.4	1403	29.79	
	8	42.4	1230	29.00	

phyrin ester were prepared, plated and counted as described. The results are given in Table III.

DISCUSSION

As seen from Fig. 1 and Table II, lead ions in a concentration of 10^{-4} Mol. inhibit the biosynthesis of heme very strongly. Table II indicates that the inhibition of the incorporation of carboxyl carbon is slightly higher then the inhibition of the iron incorporation. This apparent discrepancy could be explained by assuming that some heme has been formed from the free porphyrin present in the cell at the beginning of the incubation experiment, and the results shown in Fig. 2 strongly support this assumption. In the first 30 min. the amount of free cell porphyrin decreases and thereafter slowly but steadily increases. During the first 30 min. heme has a very high rate of formation, as shown in Fig. 1, and exceeds the rate of formation of heme precursors, causing a decrease in the concentration of free erythrocyte protoporphyrin. During the next few hours the rate of formation of heme falls off and beyond the rate of formation of porphyrin and the concentration of free porphyrin will increase.

The above results strongly indicate lead has no effect on the incorporatio iron into protoporphyrin, but mainly on the formation of the porphyrin pa the heme molecule.

The results of the paper chromatogr of the 0.1 N HCl porphyrins indicate this effect must be prior to the copro phyrin formation, if this porphyrin i intermediate in the formation of heme. is so far not proven.

Further, this experiment indicates th dicarboxylic porphyrin different from p porphyrin, probably hematoporphyrin, m be a precursor to heme.

Granick, Bogorad & Goffe (1953) suggested that hematoporphyrin is a cursor of heme and chlorophyll, probabl protoporhpyrin. This is in accordance the above findings.

The results of the present investig make it clear that the porphyrinuria in intoxication can not be due to an effe lead on the iron incorporation into prot phyrin or to an effect on the mechanis porphyrin biosynthesis.

The porhpyrinuria is most probably ondary to an effect of lead on the

or to a special effect on the por-
rming organs.
r work on lead workers and rab-
progress at the Physiological Insti-
versity of Oslo.

SUMMARY

ffect of lead on the biosynthesis of
l free porphyrins in immature rabbit
ytes and normal duck erythrocytes
studied in vitro with the aid of
cetate labeled in the carboxylgroup
and ferric ammonium citrate labeled
$^{55/59}$.
been shown that lead exercises a
hibitory effect on the formation of
t the effect is not due to a blocking
corporation of iron into protopor-
s suggested by Rimington (1938),
diminished formation of the por-
art of heme.
ggested that free erythrocyte proto-
n is used for the biosynthesis of

rboxylic porphyrin different from
phyrin has been shown to be pre-
e erythrocytes. It is suggested that
hyrin is hematoporphyrin, and that
hyrin is an intermediary step in the
of the free erythrocyte protopor-
nd thus a precursor to heme.

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THE PERMEABILITY OF THE HUMAN PAROTID GLA TO A SERIES OF SULFONAMIDE COMPOUNDS, PARAAMINOHIPPURATE AND INULIN

By S. Å. KILLMANN and JØRN HESS THAYSEN

From The Metabolic Ward of The Medical Department A, Rigshospitalet, Copenhagen, Denmark

(Received for publication July 28, 1954)

The purpose of the present investigation was to examine the permeability of the epithelium of the human parotid gland and to compare the findings to those previously reported on the sweat gland (Thaysen & Schwartz, 1953). A series of sulfonamide compounds, paraaminohippurate and inulin were chosen for the experiments and their rates of excretion in the saliva were compared to the corresponding concentrations in the plasma. The results indicate that the epithelium of the parotid gland presents a barrier to the free diffusion of solute molecules from plasma to glandular lumen. The permeability of this barrier is similar not only to that of the epithelium of the sweat gland but to that of most other cells throughout the body. A quantitative relationship appears to exist between the degree of ionization of each of the sulfonamide compounds at the physiological pH, their rates of excretion in saliva and sweat and their volumes of distribution in body water.

ANALYTICAL METHODS

Determination of non-protein bound sulfonamide and paraamino-hippurate in the plasma. 1 ml of plasma was used for the determination of "total" (protein bound and non-protein bound) sulfonamide and paraaminohippurate according to the metho Bratton & Marshall (1939). The concen tion of non-protein bound sulfonamide paraaminohippurate was determined by same method on ultrafiltrates of plasma tained by the technique of Lavietes (19 The validity of this procedure for the pre experiments was tested. Sulfanilamide quantitatively recovered from aqueous tions of 3 mg per cent when these were jected to ultrafiltration by the same techn as used for the plasma samples. This fin indicates that binding of the sulfonam to the cellophane membrane may be sidered negligible. The efficiency of the phane membranes to withhold plasma teins was demonstrated by the fact that of the ultrafiltrates showed precipit when trichloracetic acid was added to The coefficient of variation for replicate lyses on 5 ultrafiltrates of the same pl sample containing 8 mg per cent sulfa mide was 6.5 per cent.

Determination of non-protein bound fonamide and paraaminohippurate in saliva. Determination of sulfonamide paraaminohippurate in the saliva was formed in the same manner as on pl No demonstrable binding was found by filtration of saliva samples containing k

sulfathiazole. Since sulfathiazole, various sulfonamides examined, pound which is most extensively plasma proteins it was deduced g of all sulfonamides in the saliva sidered negligible. Ultrafiltration ore, not carried out routinely on amples from the different experi- e accuracy and precision of the s tested in a recovery experiment. lfanilamide was added to 50 ml saliva. The concentration in the thus 8 mg per cent. The mean on found by 5 replicate analyses g per cent with a coefficient of f 5 per cent.

e above data on replicate analyses ultrafiltrates and saliva it can be that technical errors may cause r cent variation in the ratio of the on of sulfonamide in the saliva centration of non-protein bound e in the plasma.

nation of inulin in plasma and carried out by Harrison's (1942) n of the method of Alving, Rubin 1939).

ERIMENTAL PROCEDURE

adult humans served as subjects periments. Most of them were he remainder had various minor the locomotor system, which are to interfere with water or elec- abolism.

two hours prior to a test the s given sulfonamide perorally in arying between 3 and 6 grams. eriments with paraaminohippurate the concentration in plasma was established by a priming dose of the test substance and sustained by a constant intravenous infusion.

Saliva was collected from Steensen's duct in accurately timed periods of 1 or 2 minutes duration by a method previously described (Thaysen, Thorn & Schwartz, 1954). Salivary secretion was stimulated by the subcutaneous injection of from 8 to 12 mg of pilocarpine hydrochloride in isotonic saline.

RESULTS

The excretion of sulfanilamide. Thirty saliva samples were obtained from 5 subjects with rates of salivation ranging from 0.16 to 1.95 ml per minute and concentrations of non-protein bound sulfanilamide in the plasma ranging from 1.46 to 9.60 mg per cent.

The concentration of sulfanilamide in the saliva, S, was directly proportional to the concentration of non-protein bound sulfanilamide in the plasma, P, over a 6-fold variation in P (Fig. 1). The mean ratio of S to P from all experiments was 0.87 ($\pm$ 0.10), independent of a 12-fold variation in salivary flow and independent of an 8-fold variation in the number of sulfanilamide molecules transported.

The coefficient of variation of S, after correction for the regression on P, was 12 per cent, only slightly larger than might have been expected from mere technical errors. An analysis of variance of S/P showed, however, significantly greater variance among mean values of different experiments than among consecutive values of the same experiment ($p < 0.05$). From these findings it may be concluded that the variance of S/P was due, in part, to physiological

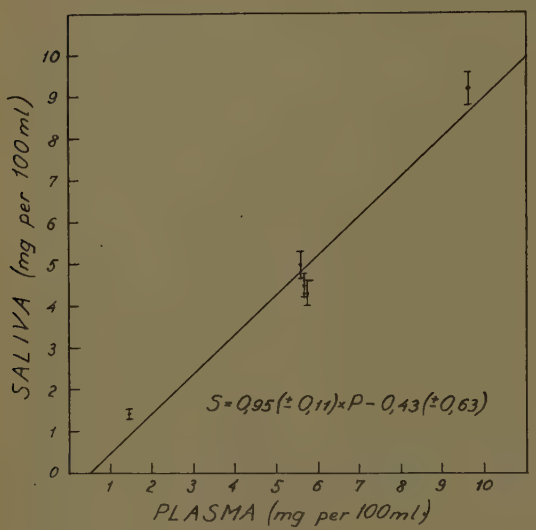


Fig. 1. The relationship between the concentration of non-protein bound sulfanilamide in plasma and in saliva. The points represent mean concentrations in saliva, the vertical lines the corresponding standard deviations.

differences between persons, but that the main source of variance was simply technical.

The excretions of methylsulfanilamide, sulfapyridine, sulfadimidine, sulfathiazole, sulfamerazine, sulfadiazine and para hippurate were tested in 12 indi experiments. The concentration in the of all substances was independent of t of salivary flow. The results are sho Table I.

The excretion of inulin was tested experiment. The inulin concentration plasma was maintained at 50 to 48 m cent throughout the experiment, h inulin could be detected in 8 samp saliva collected.

DISCUSSION

The saliva/plasma concentration ra each of the various sulfonamide com examined was unaffected by a wide of variation in the plasma concentrati in the absolute number of molecules ported. It is likely, therefore, that al pounds are excreted by a process of diffusion and not *via* a specific tra mechanism which could become sat by increasing load.

If this is so, the demonstrated diffe between S/P ratios of different com

Table I. *Saliva/plasma concentration ratios of the different sulfonamide co pounds and of paraaminohippurate.*

Compound	No. of experiments	No. of observations	Range of plasma concentration (non-protein bound) (mg per 100 ml)	Range of salivary flow (ml per min.)	Saliva/P concentr ratio
Methylsulfanilamide	1	7	2.62	0.70—1.00	0.94 (±
Sulfanilamide	5	30	1.46—9.60	0.16—1.95	0.87 (±
Sulfapyridine	2	14	1.20—2.03	0.35—1.30	0.81 (±
Sulfadimidine	2	11	3.22—3.61	0.43—1.20	0.72 (±
Sulfathiazole	2	11	1.11—2.12	0.37—1.35	0.23 (±
Sulfamerazine	2	8	3.78—4.03	0.25—0.93	0.32 (±
Sulfadiazine	2	14	0.97—4.52	0.36—2.10	0.31 (±
Paraaminohippurate	1	5	20.0	1.30—1.70	0.015(±

explained on the basis of some emical property of the sulfonamides nders the parotid epithelium un- ermeable to them. From Fig. 2 it een that a close relationship exists he S/P ratios for the various com- nd the extent to which they exist n-ionized form at the pH of plasma. ppears that the pK of the sulfona- the physico-chemical property sponsible for their differential rate tion in the saliva, and that the n of the parotid gland is permeable n-ionized molecules only. It is of o compare these findings on the pithelium with the experiments of t (1925) on single cells. By the pH of the medium Osterhout to demonstrate that the weak acid trated into the cell sap of *Valonia* n in the non-ionized form.

parison between the rates of excre- ne sulfonamides and of paraamino- in the saliva and in the sweat & Schwartz, 1953) shows several es and a difference.

milarities are the following: The tion of sulfonamide and paraamino- is independent of the secretory oth secretions. Moreover, all com- ppear to be excreted by simple both in the saliva and in the sweat. he epithelia of both glands are to the non-ionized molecules only. fference between the two secretory s that the clearances for the various s are somewhat larger in saliva weat. The saliva/plasma concen- tio for sulfanilamide is thus 0.9, /plasma concentration ratio only

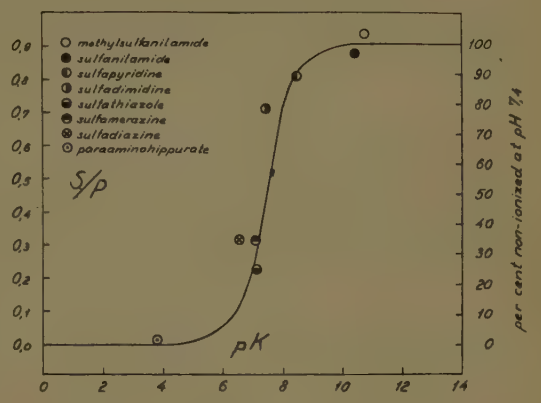


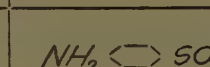
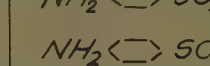
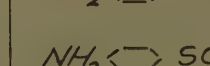
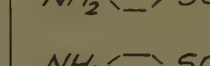
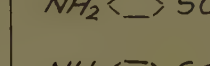
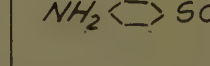
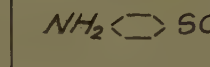
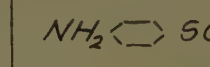
Fig. 2. The relationship between pK, S/P ratio (circles) and the percentage of non-ionized compound at pH 7.4 (solid line).

0.7. No explanation for this difference is given by the present experiments. The possibility that a difference in pH between a secretory product and the extracellular fluid may affect the concentration gradient for non-ionized molecules across the epithelial barrier was not tested. The rates of diffusion of weak electrolytes may, conceivably, differ appreciably through the epithelia of two glands that form secretions of different pH. That such a possibility is not mere speculation is indicated by the fact that the renal clearances for many sulfonamides increase with alkalinization of the urine (Beyer et al., 1944). Alkalinization of the urine will decrease the relative proportion of non-ionized sulfonamide molecules in tubular urine. Since the tubular cells are permeable to non-ionized molecules only the rate of back diffusion will consequently be reduced and the clearance increase.

In Table II a comparison is made between the saliva/plasma and the sweat/plasma concentration ratios for the different compounds

Table II. *The relationship between the saliva/plasma concentration ratio, the sw plasma concentration ratio, the volume of distribution in body water and some phy chemical properties of the various compounds examined.*

[The sweat/plasma concentration ratios were measured on man (Thaysen & Schwartz, 1953) volumes of distribution of the sulfonamides on cats (Fisher et al., 1943), and the volumes of dis tion of paraaminohippurate and inulin on man (Schwartz, 1949, 1950). The pK values o sulfonamides were determined by Bell & Roblin (1942).]

Compound	Chemical structure	Molar weight	pK	Volume of distribution (per cent of body weight)	Sweat/Plasma concentration ratio	Saliva/Plasma
N_1methyl-sulfanilamide		187	10.8	—	—	
Sulfanilamide		172	10.4	98.2	0.69	
Sulfapyridine		249	8.4	82.5	0.58	
Sulfadimidine		277	7.4	—	—	
Sulfathiazole		255	7.1	58.5	0.13	
Sulfamerazine		264	7.1	—	—	
Sulfadiazine		250	6.5	46.0	0.11	
Paraamino-hippurate		196	3.8	27.4	0.02	
Inulin		5100	—	16.2	0.00	

examined and their volume of distribution in body water. On the whole, there appears to be a close correlation between these parameters. Sulfanilamide is distributed in total body water and has a high clearance in the two secretions. Inulin maintains an exclusively extracellular localization and is excreted in neither secretion. These findings indicate that the permeability of both dular epithelia is similar to that of general. It is likely, therefore, tha stances which are neither elaborate gland nor excreted by mechanisms of transport will be transferred into a se product following general laws of permeability.

SUMMARY

liva/plasma concentration ratios for
lfanilamide, sulfanilamide, sulfa-
sulfadimidine, sulfathiazole, sulfa-
sulfadiazine and paraaminohip-
ere found to be 0.94, 0.87, 0.81,
, 0.32, 0.31 and 0.015 respectively.
substance, the S/P ratio was in-
t of large variations in the plasma
tion and in the absolute amount
les transported, indicating that all
ds are excreted by a process of
ffusion and not *via* a specific trans-
hanism which could become satu-
increasing load. If this is so, the
ated differences between S/P ratios
ent compounds must be explained
asis of some physicochemical pro-
the sulfonamides which renders the
pithelium unequally permeable to
close relationship was found be-
e S/P ratio for the different sub-
nd the extent to which they exist
n-ionized form at the pH of plasma.
cluded from this finding that the
emical property mainly responsible
ifferential rate of excretion of the
sulfonamides is their pK and that
id epithelium is permeable to the
ed molecules only. A close correla-
found between the saliva/plasma
tion ratio, the sweat/plasma con-
n ratio and the volume of distribu-
body water of the compounds
, showing that the demonstrated
ity characteristics of the parotid
n are common to cells in general.
dings indicate that substances which
er elaborated in a gland nor ex-
processes of active transport will
be transferred into the secretory product following general laws of cellular permeability.

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THE DETERMINATION OF SERUM CALCIUM

A COMPARISON BETWEEN THE METHOD OF CLARK AND COLLIP AND THE TITRATION WITH ETHYLENEDIAMINE TETRA-ACETATE

By L. ELDJARN, O. NYGAARD* and S. L. SVEINSSON

From Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Nor and Central Laboratory, Ullevål Hospital, Oslo, Norway

(Received for publication Nov. 15, 1954)

Several methods have been described for the determination of serum calcium by chelating agents, notably ethylenediamine tetra-acetate (EDTA), in the presence of a suitable calcium-indicator (for references, see Fales and Lehmann 1953). For some time we have compared the results obtained by the standard method of Kramer & Tisdall (1921) as modified by Clark & Collip (1925) with the results obtained by titrating with EDTA in the presence of ammonium purpurate in strongly alkaline medium. The procedure we have been using for the EDTA titration is essentially a simplification of the procedure described by Fales, and in some respects it resembles the second method described by Lehmann.

EXPERIMENTAL

The standard method of Kramer & Tisdall as modified by Clark & Collip was used by an experienced medical technologist. *The EDTA titration method* was carried out as follows: In a large Coleman spectrophotometer tube one ml of serum (or Ca^{++} standard) is mixed thoroughly with 9 ml of 0.2 *N* NaOH and 1 ml of a solution containing 1 mg of ammonium purpurate[1] ml. The tube is placed in the spectrop meter, previously set to a wave leng 600 mμ, and the extinction is adjust zero.

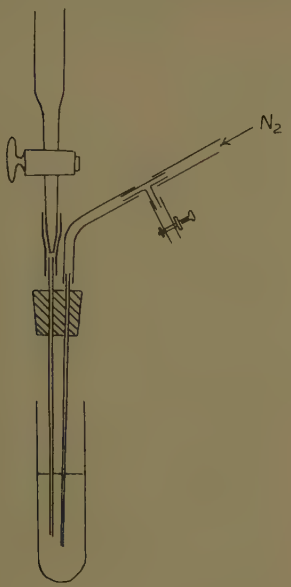
To the sample is added 0.5 ml or 1 portions of a solution containing 0 EDTA per l and the extinction is each determined after mixing of the contents EDTA is added from a burette supplied a capillary extension which is inserted the solution during the addition of th agent. The mixing is obtained by bub nitrogen through a second capillary s taneously inserted (Fig. 1). To pr excessive foaming of the serum sampl few drops of amyl alcohol are added to tube.

The addition of EDTA is continu about 2 ml beyond the point where extinction values begin to fall off.

The extinction values are plotted function of the amount of EDTA a Lines are drawn through the last three of the ascending part of the curve through the first two or three points descending part, the point of maxi extinction being excluded. The inters between these two lines represents th point for the titration. The equivalen the EDTA solution was determined for set of serum analyses.

[1] Ammonium purpurate obtained from several manufacturers, and of varying degree of purity, has been tried and found equally satisfactory.

* Fellow of the "Norwegian Cancer Society", Oslo, Norway.



Arrangement for the titration og serum calcium with EDTA.

ents on the EDTA titration method.
ove procedure no correction is made
dilution of the solution due to the
of EDTA or for the fading of the
to the photosensitivity of the pur-
since, however, the extinction values
ve to determine the titration end
ank readings are unnecessary pro-
t the titration is carried out quickly
mixing of the reagents and that
es are not exposed to intense, direct
ng the titration period.
resence of magnesium ions in the
tion range 0—10 mg per cent was
to interfere with the titration end
longed contact of the 0.2 *N* NaOH
must be avoided, since it was found
an apparent decrease in the equiv-
the EDTA solution. Sodium sili-
not interfere with the titration.
ure variation between 10°—30° C
l to be without effect on the titra-
point.

RESULTS

When comparing the values obtained with the two methods, the EDTA titration gave on the average lower values than those obtained by the Clark & Collip method. Lehmann found a constant difference (0.4 mg per cent) between the two methods. In our case, however, the best agreement is obtained when the EDTA values are multiplied by a factor, which in our series was found to be 1.046 (standard deviation $\pm$ 0.030). In Fig. 2 are plotted the results obtained with the two methods on 82 serum samples, the abscissa being the result with the Clark & Collip method and the ordinate the results with the EDTA titration after multiplying with the correction factor (1.046).

The reproducibility of the two methods was determined by comparing the average difference between duplicates: For the Clark & Collip method the average spread for 82 sets of duplicates was 0.09 mg per cent; in

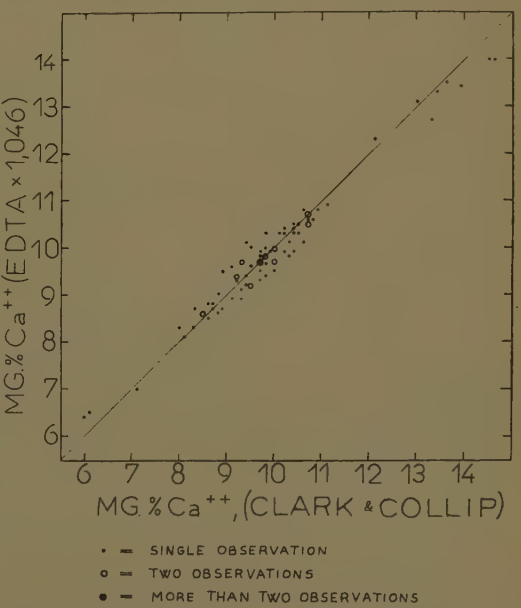


Fig. 2. A comparison between the results obtained with the Clark & Collip method and the result with the EDTA titration after the introduction of the correction factor (1.046).

6 cases out of 82 the spread was greater than 0.2 mg per cent. For the EDTA method the average spread for 61 sets of duplicates was 0.120 mg per cent and in 8 cases the spread was greater than 0.2 mg per cent. The reproducibility of the two methods is thus approximately the same. For the purpose of routine work we have found that titration with EDTA is on the average twice as fast as the Clark & Collip method. Furthermore, the EDTA method has the added adva that it is able to give the answer for a cular sample within a matter of minutes.

SUMMARY

A comparison has been made betw standard clinical method for the deter tion of serum calcium (Clark & Collip a modified method for the titration ethylenediamine tetra-acetate (EDTA

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Lehmann, J.: A photoelectric micro method f direct titration of calcium in serum ethylenediamine tetra-acetate. Scandina Clin. & Lab. Investigation, *5*, 203, 1953

ARKS ON THE CENTRIFUGAL FORCE NECESSARY . OR SEPARATION OF PLASMA FOR FIBRINOGEN DETERMINATION

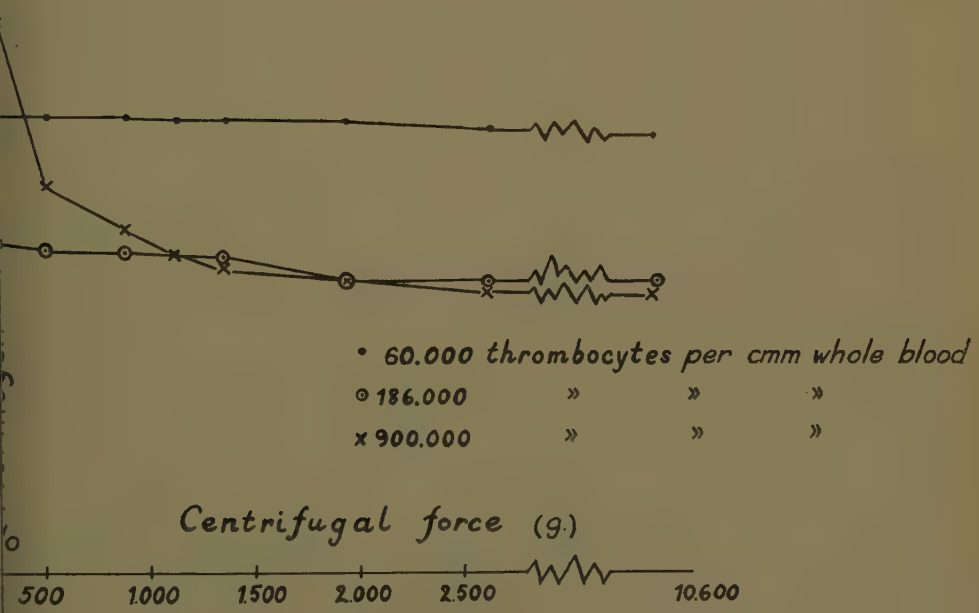
or determination of fibrinogen is llected with citrate or oxalate as gulant. Recently ethylene-diamino- c acid (EDTA) has become a nticoagulant and will probably be rally for blood-sampling, as this does not interfere with most nalyses.

sma is separated from the blood by centrifugation. In most ised, the fibrinogen is then con- to fibrin by recalcification or by thrombin. Any thrombocytes pre- e plasma sample will be occluded in the clot and thereby give fictiously high fibrinogen values. This error may be considerable, especially if the blood is collected with the sodium salt of EDTA as an anticoagulant, as the thrombocytes are well preserved in EDTA-blood.

EXPERIMENTAL

Blood was run directly into centrifugal tubes containing 5 mg Na_2 EDTA per ml collected blood.

The samples were centrifugated 10 minutes in a centrifuge with 200 g at the top and 300 g at the bottom of the centrifugal tubes.



The plasma, practically free of red cells, was sucked off and re-centrifugated at different g values. In the calculation of g the radius (R) as measured from the center of rotation to the middle of the plasma column was used. The conical tubes (15 ml) were provided with 3 ml of this plasma and re-centrifugated for 10 minutes.

The centrifugal force used was calculated by the usual formula $g = 0.00001117 \times R \times (r.p.m.)^2$.

Some typical results are recorded in Fig. 1.

Plasma containing varying amounts of thrombocytes were chosen.

From the figure it is evident that the fibrinogen values are relatively independent of the centrifugation velocity used for separation of plasma when the thrombocyte of the blood is subnormal, but that high centrifugation was necessary for sepa of plasma from patients with abnormally thrombocyte values. Low speed centri tion, less than 1000 g, results in about cent too high values when analysing p from individuals with normal amou thrombocytes in whole blood. The er much greater when the amount of thro cytes is increased. These curves and obtained from studies of patients with n thrombocyte values showed that centri tion with at least 2000 g ought to be re mended for the separation of plasm determination of fibrinogen when Na_2E is used as an anticoagulant.

Department of Clinical Chemistry,
Malmö General Hospital, Malmö, Sweden.
Jan. 9, 1955.

Carl-Bertil Laur

ELET ACCELATOR: IDENTICAL TO PROACCELERIN AND ADSORBED FROM PLASMA?

8 Ware, Fahey & Seegers reported accelerator (platelet factor 1. Ste-53) acting like *serum* Ac-globulin. n centrifugation experiments they at this factor and the serum Ac- are "entirely different proteins". hry & Seegers (1950) believe this be "an integral part of the platelets". ve found that a suspension of nor-shed platelets has a *proaccelerin-* Ac-globulin-) like activity, which ightly reduced by 10 washings. This ctivity accounts for about per cent accelerin activity of normal, citrated The proconvertin and prothrombin of suspensions of normal, washed are very low.

rin or serum Ac-globulin is defined oagulant activity produced by the of proaccelerin (plasma Ac-globu-rombin. By adding a small amount in to a platelet suspension the acce-ctivity increased about tenfold, any reduction in platelet number. increase in activitiy was also found et extracts. This effect is of the der as that produced by adding to proaccelerin (plasma Ac-globu-e platelet accelerator therefore be-e *pro*accelerin.

ts from a patient with congenital roaccelerin (parahemophilia, Owren owed only about 1/50 of the accele-vity of normal platelets. However, ese platelets were incubated with platelet-poor plasma followed by repeated washings, their accelerator activity increased to normal. Parahemophilia platelets therefore do adsorb proaccelerin from normal plasma, and then behave in a manner similar to normal platelets.

Trypsin destroys the proaccelerin in plasma. (Small amounts give a partial activation to accelerin). When a suspension of normal, washed platelets was incubated with trypsin, 90 per cent or more of the accelerator activity was destroyed, without any reduction in platelet count. When these platelets were washed and incubated with normal, platelet-poor plasma and again washed, they also regained a normal accelerator activity by adsorption from the plasma.

The platelet accelerator is labile on storage. During storage a partial activation to accelerin may take place, depending on the conditions of storage. The platelet accelerator is destroyed by heating to 53° C for 20—30 min. Like Ware et al. (1948) we have found that 80 per cent or more of the platelet accelerator activity is sedimented when a platelet extract is centrifuged for 30 min. at 32,000 g. We believe this is due to the platelet accelerator being adsorbed on platelet fragments.

The platelet accelerator plays an important role in the clotting theories of Seegers, Tocantins and Stefanini (cit. Albritton 1952) as a catalyst of the initial formation of thrombin. This role can not be a decisive one, because the thromboplastin time is very little influenced by the presence of platelets.

Based on the evidence given we believe that the platelet accelerator activity is caused by plasma proaccelerin which is adsorbed on the platelets.

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Department of Medicine, University Hospital, Rikshospitalet, Oslo, Norway.
Febr. 20, 1955.

Peter Hjort,
Samuel I. Rapapo
Paul A. Owren.

ECTROPHORETIC ANALYSIS OF HUMAN ACUTE PHASE PROTEIN

ppearance of acute phase protein
ve protein) in different diseases has
stigated by several authors (Tillett
cis 1930, Ash 1933, Löfström 1943,
1947). In 1943, Perlman, Bullowa,
lkind presented results indicating
C-reactive protein follows the α-
group in moving boundary electro-
Their conclusion was based on
vidence only, and some contradic-
ngs remained unexplained; there-
ation of the investigation by some
thod seemed to be indicated. The
is a report of an examination of
se serum by continuous zone elec-
is as described by Svensson and
1949.

from two patients in the acute stage
ococcal pharyngitis and maxillary
respectively, were investigated. The
pooled immediately before the frac-
diluted to a protein concentration
cent, and dialyzed against a veronal
the ionic strength 0.027 and pH
ml of whole serum was fractionated
ffer with potato starch as the sup-
rial. The initial zone breadth was
the rate of fractionation 0.8 ml of
um per hour. A current of 245 mA
ied, giving a cell temperature of
he equipment used has been de-
Brattsten 1952, 1955. The distri-
the protein has been recorded in
means of the optical density at

a submitted to fractionation and the
were tested for acute phase protein
psular swelling reaction described
m 1943. Pneumococci suspensions
3 B were used. The original sera

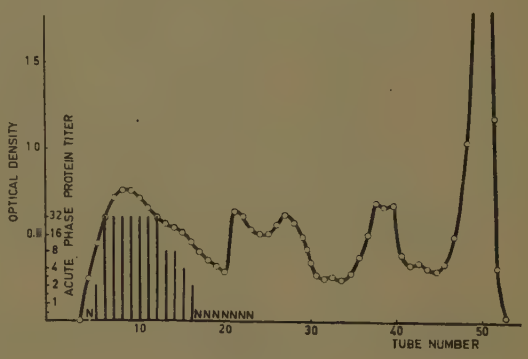

Fig. 1.

showed a titer of 16. This value did not change during the dialysis against the veronal buffer. For the analyses of the fractions, samples corresponding to 2—3 ml of whole serum were dialyzed against Ringer's solution and concentrated by pervaporation in cellophan bags to a final volume of 1 ml.

In order to localize the acute phase protein in relation to the main electrophoretic components (see Fig. 1) eight pools were prepared of the fractions 4—13, 14—19, 20—23, 24—31, 32—35, 36—41, 43—47, 48—53, ten ml of each fraction entering in the respective pools. The material was then concentrated as described above. All the resulting solutions were negative except that containing the fractions 4—13, the titer of which was as high as 32. This result showed that the reactive substance resided in the γ-globulin fraction of the sera investigated.

On the basis of this result each 15 ml of the fractions 4—23 were analyzed separately. The titration values are presented in Fig. 1. The highest titer, 32, was found in the fractions 6—12, which correspond to the maximum in the γ-globulin distribution curve.

The correlation between the titration and optical density records in this region suggests that the reactive substance has a fairly even distribution among the γ-globulins, occurring in the fractions in approximate proportion to the total protein concentration.

As a check of the validity of these findings, the original serum and fraction No. 9, found to contain acute phase protein, were analyzed together by paper electrophoresis. The experiment established the fact that the active fraction contained protein migrating with the mobility of a γ-globulin exclusively.

Further details of the investigation will be reported in a forthcoming paper.

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Per He
Inger Bratts

The Hospital for Contagious Diseases
and State Bacteriological Laboratory,
Stockholm, Sweden.
The Institute of Biochemistry,
University of Uppsala,
Uppsala, Sweden.
March 13, 1955.

Book Reviews

TTI: *Porphyrins. Their biological chemical importance.* Hilger & tts, Ltd., Hilger Division. London, 4. 50 shillings.

which Professor Vannotti has written not only a revision and translation of own monograph "Porphyrine und Porheiten" which was published in 1937, lly a completely new book which inmodern views on pigment metabolism. has received the best possible recomn that another of the world's most phyrin investigators, Professor C. Rimdon, has made the translation. In a Foreword this distinguished scientist fessor Vannotti's book is a challenging the fundamental relationships of the to the life of the cell in normal and conditions, and is enriched by the inical observation which he is able to from his own experience as head of ity Polyclinic at Lausanne."

S. L. S.

: *Myokardstoffwechsel und Herzapie.* Georg Thieme Verlag, Stutt-, 1954. D. M. 29.70.

l's book provides an excellent review blogy, pathology and clinical aspects of considerable quantity of valuable inassembled within the relatively modest 0 pages. The physiological and pathows are treated throughout in connection considerations of the therapeutic posported by personal clinical observations. ochemical point of view it is a pleasure clear and concise presentation in the chapter on the importance of the energy-rich phosphorus compounds for myocardial function. Carbohydrate metabolism is also well presented, although it may be justified to accept some of the therapeutic conclusions drawn by the author with some hesitation.

The bibliography is extensive, but unfortunately not alphabetically arranged. The subject index is excellent. The book seems to be a very valuable contribution to the literature on cardiology.

F. L.

G. Asboe-Hansen: *Connective tissue in health and disease.* Ejnar Munksgaard, Copenhagen, 1954. 50 Danish Crowns.

In 320 pages the author has collected, contributions from more than twenty scientists engaged in connective tissue research. These contributions cover a vast and varied field, but seem well adapted to form a comprehensive symposium. The idea of the book is to make it possible for any doctor to be acquainted with "the status and significance of connective-tissue science". The book thus forms an urgent contribution to our medical library.

This field, which until a few years ago was practically unknown to all but a few, is now of primary importance in medicine. One has only to point to the significance of the spreading factor of connective tissue, of hyaluronidase, of mucopolysacharides and arteriosclerosis, of the mechanism of the action of cortison and ACTH, to give an idea of a few of the many problems treated. Most of the contributions are presented by the leading men of modern connective tissue research, and this of course also adds to the pleasure all interested in medical science will obtain through the reading of Asboe-Hansen's book.

E. B.

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